A HANDBOOK OF COLORIMETRIC CHEMICAL ANALYTICAL METHODS

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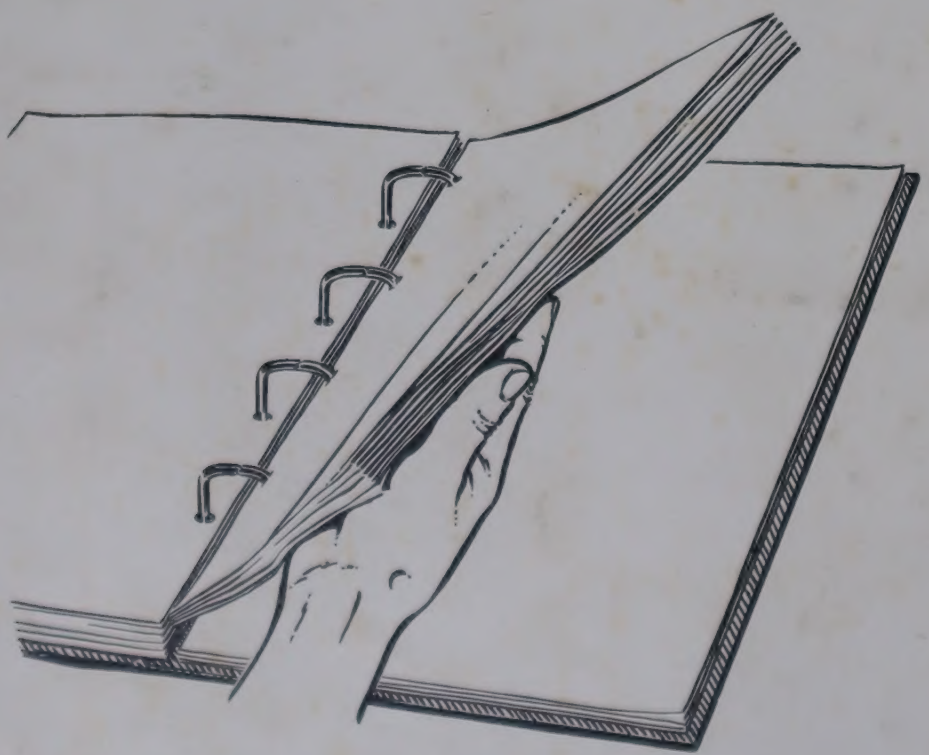

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Colorimetric Chemical Analytical Methods

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Key to the use of this book

Alphabetical Index : Tests are listed under the name of the test, and also under the subject matter and class of work or industry. Against each is shown the Part of the book in which the test is placed. In each Part the tests are arranged alphabetically.

Summary of Tests : At the beginning of each Part are listed the tests included, together with a note of which instrument is needed, and what other apparatus is required.

Index of Parts of the book : For ease in reference, the book has been divided into seven Parts. Tests which are appropriate to more than one are repeated in each appropriate Part. Each Part has a different colour as a key. The appropriately coloured cover commences that Part, and the pages have a coloured corner to identify them. The colours are as follows :—

PART 1. Lovibond Apparatus

PART 2. *p*H

PART 3. Chemical Analysis—Inorganic

PART 4. Chemical Analysis—Organic

PART 5. Chemical Pathological Methods

PART 6. Noxious Vapours

PART 7. Miscellaneous—Colour Grading for Quality

Introduction

COLORIMETRIC CHEMICAL ANALYSIS is defined as the determination of the amount of a particular chemical which is present in a sample, using a chemically developed colour as a measure of the concentration. This method of analysis requires three separate procedures :

1. The isolation of the chemical to be determined from any interfering materials which may be present in the sample
2. The production of a colour by the action of some chemical reagent upon the isolated chemical
3. The measurement of the colour so produced.

These three processes are quite distinct and will therefore be discussed separately.

1. ISOLATION of the material to be estimated depends to a large extent on the nature of the sample in which it occurs and on the particular chemical ions which will interfere with the quantitative method to be used. Each individual determination therefore poses separation problems for which there can be no universal answer. The most that can be done to help the analyst is to indicate the general methods which can be used for getting material into solution; to state in the individual test procedures any solution method which cannot be used; and to refer the reader to published papers on specific applications of determinations, which contain details of the methods used for getting the samples into solution.

In many applications of colorimetric chemical analysis the problem of getting the sample into solution does not arise as the sample itself is a liquid.

This is the case in the examination of water samples, the examination of trade effluents, quality control by examination of a liquid product, examination of body fluids, etc., etc. Such applications cover roughly 50% of the analyses for which the tests described in this book were originally devised.

Even in these cases however it may be necessary to remove some interfering substances before developing the test colour. Such removals can be effected by the use of absorbents such as activated charcoal; by ion exchange resins; by column chromatography; or by developing the analytical colour in the presence of the impurity and then selectively extracting the colour with a solvent before estimation. Where the use of such techniques is required, then specific mention of the fact is made in the test procedure.

An alternative technique which is also widely used in the original tests is that of extracting the material to be determined from the sample matrix by suitable solvent procedures. No single method of extraction is suitable for use in every case and individual reference to the preferred techniques is made in the appropriate test techniques.

In many cases, especially in metallurgical analysis, it is possible to dissolve the sample fairly readily by the use of an acid or a mixture of acids. The acid used and the optimum concentration of acid will vary according to the chemical nature of the sample. However, provided that care is taken not to use an acid the anions from which will interfere with the subsequent colorimetric reaction, or which will destroy the ion to be determined; and provided that the final pH is adjusted to its optimum value for that particular determination, then any procedure which will dissolve the sample can be adopted safely.

An extension of this acid solution technique is that of "wet ashing."* This method of solution is particularly useful when small amounts of metal ions have to be determined in organic matter and it is essential to destroy completely the organic material.

In this method of solution the sample is digested with a mixture of sulphuric, nitric and/or perchloric acids, sometimes in the presence of a catalyst, until the solution becomes colourless. The sulphuric acid is then evaporated and the residue heated until copious white fumes are evolved. The residue is subsequently taken up in water, neutralised if necessary, and treated with the appropriate colorimetric reagent.

Dry ashing is also sometimes employed, usually as a method of concentration prior to chemical solution. This technique also is limited to the determination of metal ions, which are not destroying by ashing. Great care must be taken when using this technique that none of the material is lost by evaporation, or by sputtering. To avoid the loss of the more volatile elements, ashing should be carried out at as low a temperature as possible (400–450°C) and air-flow through the furnace should be limited to the minimum required to complete oxidation. It is sometimes preferable to prepare a 'sulphated ash' and this is done by carefully adding sulphuric acid to the initial ash and reheating, repeating the procedure until all traces of carbon have disappeared from the ash.

2. COLOUR PRODUCTION is carried out by chemically coupling the ion to be determined, if it is not already coloured, with a suitable chromophoric group. Many colorimetric reagents are now available for this purpose **and virtually any substance can now be determined colorimetrically by the use of the appropriate reagent.** The reagents used for the determination of specific ions are described in the appropriate test procedures. It must be emphasised that these are only those reagents for which discs have been calibrated in connection with specific procedures. Tintometer Ltd. are always ready to produce further discs for use with alternative reagents and to advise on the appropriate reagent for use in particular circumstances.

3. MEASUREMENT OF THE COLOUR produced by the colorimetric reagent may be carried out by either visual or photo-electric photometry. The eye is still the most sensitive and accurate means of comparing colours, and this book is limited to visual colorimetric procedures. Some people have an entirely erroneous picture in their minds of the incidence of 'colour blindness' in individuals, and for this reason distrust the use of visual colorimetric methods. 'Colour blindness' in its extreme form of insensitivity to every colour is almost unknown. Some form of colour deficiency, including cases of mild colour confusion, occurs in approximately 8% of males and 0.5% of females. All others are colour normal and are capable of a very high order of colour discrimination. In addition, the colour deficiency of a large proportion of those whose colour vision is in some way defective is not sufficiently marked to render them incapable of carrying out colorimetric analysis. Further, it is equally dangerous to use those who prove to be incapable of adequate visual colour discrimination for colorimetric analysis using a photo-electric photometer. The photo-electric instrument, it must be remembered, cannot 'see' colour and it relies on its human operator to 'see' that the solution being measured is of the correct colour.

The visual measurement of colour is carried out by matching the test colour against a range of predetermined colour standards. It would obviously be impracticable to have every possible colour in any range of individual standard colours, and a method has therefore been devised whereby any colour can be matched by a mixture of standards of the three primary colours, red, yellow and blue. This method, using the **Lovibond Colour Scale,** was devised about 1885

*See "Methods for destruction of organic matter," *Analyst*, 1960, **85**, 643

by J. W. Lovibond. The scale consists of three series of unfadeable coloured glass slips, one of red (magenta), one of yellow and one of blue. Each series is a linear scale of the same colour, increasing by equal steps from the palest shade perceptible to a fully saturated (spectral) colour, and is divided into units and decimals of a unit. The colours are permanent and are exactly reproducible by the makers (the Tintometer Limited, of Salisbury, England). By superimposing suitably chosen glasses, any colour can be matched—there are, in all, nine million possible combinations of these glasses—including a grey series down to black. All possible colours can in this way be recorded in terms of three numbers (e.g. "a" units Red, "b" units Yellow, "c" units Blue) and reproduced from these figures at any laboratory which has access to a Tintometer or to a set of Lovibond glasses.

Lovibond units of colour have received international recognition, and standardising bodies in over 20 different countries quote colours in terms of units of the Lovibond Scale.

The Lovibond Scale has also been related to the **COMMISSION INTERNATIONALE DE L'ECLAIRAGE** system of colour specification (R. K. Schofield, *J. Sci. Inst.*, **16,** 1939, 74) so that records are interchangeable between the two systems. A specification expressed in C.I.E. chromaticity co-ordinates can thus be converted into Lovibond units, assembled, and looked at as an actual sample of the colour.

In chemical colorimetry it is usually most convenient to match the colour produced in the test against a series of colour standards representing known amounts of the compound being determined. In photoelectric colorimetry this series of standards is frequently reduced to a calibration curve obtained by plotting absorbance at a given wavelength against concentration; it is however basically the same matching procedure, but carried out instrumentally over a more limited range of wavelengths. For the purpose of visual photometry, instead of reducing the series of standards to a series of absorbance figures, the colours are measured in Lovibond units and reproduced as permanent glass standards using the Lovibond glasses. For convenience, these standards are mounted in discs for use either in Lovibond Comparators or in B.D.H. Lovibond Nesslerisers.

These standards are available as an exact match of the standard solutions prepared by skilled workers in the laboratories of specialists, in many different parts of the world, who co-operate with Tintometer Limited. The standards are then checked and cross-checked in other laboratories. Being absolutely permanent, the colours can be relied upon indefinitely and thus not only save time previously spent in preparing fresh chemical standards, but also simplify procedure and give confidence. Any error in carrying out the test is immediately apparent from an inability to match the solution against the standards, and the operator is able to institute an immediate check of the reagents and technique. This is in marked contrast to the situation in photoelectric colorimetry, where errors will go undetected until the operator inspects the colour visually and compares it with a reliable visual standard.

This book contains details of many chemical tests which have been specially developed for use with Lovibond colour standards. Although the tests have frequently been developed with a specific application in mind, it must be stressed that ultimately all colorimetric tests reduce to the measurement of a colour in a solution from which all possible interfering colours have been removed. The application of these tests to other materials is thus merely a question of adequate prior treatment of the sample and it is to assist in this that these introductory notes have been written. New test details are continually being added to this book and the procedures of old tests revised. The book has been designed in loose-leaf form so that constant revision can be carried out with the minimum of inconvenience. Enquiries concerning tests or test applications not mentioned in the book will be welcomed by the publishers, Tintometer Limited, Salisbury, England.

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Classification	Test	Part	Colour Code
Asphalt	Quality Grading of Refined Lower Boiling Products of Coal Tar	7	Dark Blue
	Colour Grading of Sand for Quality	7	„ „
	Determination of Phenols	4	Orange
Atmospheric Polution (See Toxic Gases)			
Bacon Curing	Determination of Nitrate	3	Brown
	Determination of Nitrite	3	„
	Determination of Copper	3	„
Baking	Estimation of Humidity	7	Dark Blue
	Quality Testing of Fats and Oils for Rancidity	7	„ „
Baths (See Swimming Pools)			
Benzole	Determination of Carbon Disulphide	4	Orange
	Determination of Sulphur	3	Brown
	Determination of Thiophen	4	Orange
	Colour Grading of Benzole and Allied Products	7	Dark Blue
Beer (See Alcoholic Drinks)			
Beverages (Non-Alcoholic)	Determination of Lead (Tea)	3	Brown
	Determination of Copper (Fruit Juices)	3	„
	Determination of Lead (Fruit Juices)	3	„
	Determination of Furfuraldehyde (Coffee)	4	Orange
	Colour Grading of Water (Mineral Waters)	7	Dark Blue
Bleaching and de-colourising	Determination of Chlorine	3	Brown
	Determination of Sulphite	3	„
	Determination of Activated Carbon	7	Dark Blue
	Determination of Formaldehyde	4	Orange
Boards	Determination of Sulphite	3	Brown
	Determination of Formaldehyde	4	Orange
Boiler Water	Determination of Amines	4	„
	Determination of Ammonia	3	Brown
	Determination of Chromium	3	„
	Determination of Hydrazine	4	Orange
	Determination of Oxygen	3	Brown
	Determination of Manganese	3	„
	Determination of Phosphate	3	„
	Determination of Silica	3	„
	Determination of Sugar	4	Orange
	Determination of Iron	3	Brown
	Determination of Sulphite	3	„
	Colour Grading of Water	7	Dark Blue
	Determination of Nitrate	3	Brown

Classification	Test	Part	Colour Code
Brewing (See Alcoholic Drinks)			
Bottle Washing	Determination of Chlorine	3	Brown
	Determination of Chlorine Dioxide	3	,,
	Determination of Detergents	4	Orange
Building	Determination of Sulphate	3	Brown
	Quality Grading of Sand	7	Dark Blue
	Determination of Copper	3	Brown
	Estimation of Humidity	7	Dark Blue
Canning	Determination of Nitrate	3	Brown
	Determination of Nitrite	3	,,
Ceramics	Determination of Titanium	3	,,
Chicken Houses	Determination of Ammonia	3	,,
Cheese-Making	Quality Grading of Milk	7	Dark Blue
	Determination of Penicillin in Milk	7	,, ,,
	Determination of Phosphatase	7	,, ,,
Coal and Coal Products	Determination of Total Phenols	4	Orange
	Colour Grading of Benzole and Allied Products	7	Dark Blue
	Quality Grading of Refined Lower Boiling Products of Coal Tar	7	,, ,,
	Colour Grading of Refined Cresylic Acid	7	,, ,,
	Determination of Cyanide	4	Orange
Clinical	Determination of Ammonia in Urine	3	Brown
	Determination of Bilirubin	5	Green
	Determination of Bromide	5	,,
	Approximate determination of Carboxyhaemoglobin	5	,,
	Determination of Chloroquine	5	,,
	Determination of Chloroform	4	Orange
	Determination of Cholesterol	5	Green
	Determination of Cholinesterase	5	,,
	Determination of D.D.T.	4	Orange
	Determination of D.D.S.	5	Green
	Determination of D.N.O.C.	5	,,
	Determination of Haemoglobin	5	,,
	Lange's Colloidal Gold Test	5	,,
	Determination of Lead	3 and 5	,, ,,
	Determination of Mepacrine	5	,,
	Determination of Morphine	5	,,
	Determination of Nicotinic Acid	4	Orange
	Determination of Paludrine	5	Green
	Determination of pH of Blood	5	,,
	Determination of Phenylpyruvic Acid	5	,,
	Determination of Phosphatase	5	,,
	Determination of Phosphate	5	,,

Classification	Test	Part	Colour Code
Clinical—*continued*	Determination of P.A.S.	5	Green
	Determination of Proteins	5	,,
	Determination of Sugar	5	,,
	The Sulphobromophthalein Test	5	,,
	Determination of Sulphetrone	5	,,
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Coffee (See Beverages)			
Cosmetics	Determination of Bismuth	3	Brown
	Determination of Bromide	3	,,
	Determination of Copper	3	,,
	Determination of Lead	3	,,
Dairies (See Milk and Milk Products)			
De-Mineralised Water	Determination of Silica	3	,,
Distilling (See Alcoholic Drinks)			
Drainage (Roads)	Determination of Detergents	4	Orange
	Colour Grading of Refined Cresylic Acid	7	Dark Blue
	Determination of Phenols	4	Orange
Drinking Water	Determination of Aluminium	3	Brown
	Determination of Ammonia	3	,,
	Determination of Bromine	3	,,
	Determination of Chlorine	3	,,
	Colour Grading of Water	7	Dark Blue
	Determination of Fluoride	3	Brown
	Determination of Iron	3	,,
	Determination of Dissolved Oxygen	3	,,
	Determination of Phenols	4	Orange
	Determination of Silica	3	Brown
	Determination of Sulphate	3	,,
	Determination of Vanadium	3	,,
	Determination of Copper	3	,,
	Determination of Lead	3	,,

Classification	Test	Part	Colour Code
Drugs	Determination of Bromide	5	Green
	Determination of Chloroquine	5	,,
	Determination of 4:4'-Diamino-Diphenylsulphone (DDS)	5	Green
	Determination of Mepacrine	5	,,
	Determination of Morphine	5	,,
	Determination of Paludrine	5	,,
	Determination of Sulphetrone	5	,,
	Determination of Suramin	5	,,
Dry Cleaning	Determination of Carbon Tetrachloride	4	Orange
Dye-stuffs	Determination of Tin	3	Brown
	Determination of Formaldehyde	4	Orange
Eggs	Determination of *a*-Amylase (2)	7	Dark Blue
Edible Oils	Determination of Furfuraldehyde	4	Orange
	Quality Testing of Fats and Oils for Rancidity	7	Dark Blue
Electro-Plating	Determination of Chromium	3	Brown
	Determination of Copper	3	,,
	Determination of Copper Sulphate	3	,,
	Determination of Cyanide	4	Orange
	Determination of Nickel	3	Brown
Effluents	Determination of Phenols	4	Orange
	Determination of Cyanide	4	,,
	Determination of Formaldehyde	4	,,
	Determination of Copper	3	Brown
	Determination of Chromate	3	,,
	Determination of the Biochemical Oxygen Demand (B.O.D.)	7	Dark Blue
	Determination of Oxygen	7	Brown
	Determination of Ammonia	3	,,
	Determination of Nitrate	3	,,
	Colour Grading of Water	7	Dark Blue
Fats	Determination of Nickel	3	Brown
	Determination of Copper	3	,,
	Quality Testing of Fats and Oils for Rancidity	7	Dark Blue

Farming (See:—
Animal Feedingstuffs, Agriculture
Antibiotics
Cheese Making
Chicken Houses
Milk
Eggs
Effluents
Soil Analysis)

Fertilisers
(See:—Soil Analysis)

Classification	Test	Part	Colour Code
Fibres (Jute, Cotton, Silk)	Determination of Dichlorophene	4	Orange
	Determination of Pentachlorophenates and Pentachlorophenol	4	,,
	Determination of Copper	3	Brown
	The Colour Grading of Resins	7	Dark Blue
Foam Rubber	Determination of Iron	3	Brown
	Determination of Tin	3	,,
Fruit Juices (See:—Beverages)			
Fumigation	Determination of Formaldehyde	4	Orange
	Determination of Cyanide	4	,,
Foodstuffs (See also:— Bacon Curing Baking Beverages Bottle Washing Fats Canning Cheese Making Milk Eggs Ham Curing Edible Oils Meat Processing)	Determination of Carbohydrates	4	Orange
	Determination of Carotene	4	,,
	Determination of Cobalt	3	Brown
	Determination of Copper	3	,,
	Determination of D.D.T.	4	Orange
	Determination of Detergents	4	,,
	Determination of Formaldehyde	4	,,
	Determination of Lead	3	Brown
	Determination of Nicotinic Acid	4	Orange
	Determination of Nitrate	3	Brown
	Determination of Nitrite	3	,,
	Determination of Tin	3	,,
	Determination of Vitamin A	4	Orange
	Colour Grading of Caramel Solutions	7	Dark Blue
	Determination of Nickel	3	Brown
	Determination of Sulphite	3	,,
	Determination of Nicotine	4	Orange
	Quality Testing of Fats and Oils for Rancidity	7	Dark Blue
Fuel (Oils and Gas)	Determination of Carbon Disulphide	4	Orange
	Determination of Sulphur	3	Brown
	Determination of Thiophen	4	Orange
	Determination of Pyridine	4	,,
	Determination of Silica	3	Brown
Fungicides	Determination of Formaldehyde	4	Orange
	Determination of Pentachlorphenol	4	,,
	Determination of Dichlorophene	4	,,
Galvanised Tanks	Determination of Copper	3	Brown
Gardening (See:—Soil Analysis Agriculture)			
Grain Storage	Estimation of Humidity	7	Dark Blue
Grass (Dried)	Determination of Carotene	4	Orange

Classification	Test	Part	Colour Code
Ham Curing	Determination of Nitrate	3	Brown
	Determination of Nitrite	3	„
Horticulture (See:— Soil Analysis Agriculture)			
Hydroponics (See:—Soil Analysis Agriculture)			
Humidity	Estimation of Humidity	7	Dark Blue
Ice Cream (See:—Foodstuffs)			
Industrial Wastes (See:—Effluents)			
Insecticides (See:—Pest Control)			
Jam (See:—Foodstuffs)			
Lacquer	Determination of Formaldehyde	4	Orange
	Determination of Sulphite	3	Brown
	Colour Grading of Resins	7	Dark Blue
Latex	Determination of Manganese	3	Brown
Laundries	Determination of Chlorine	3	„
	Determination of Iron	3	„
Leather	Determination of Pentachlorphenates	4	Orange
	Determination of Chromium	3	Brown
Lubricating Oils (See:—Oils)			
Meat Processing	Determination of Copper	3	„
	Determination of Lead	3	„
	Determination of Nitrate	3	„
	Determination of Nitrite	3	„
	Determination of Formaldehyde	4	Orange
Metallurgy	Determination of Aluminium	3	Brown
	Determination of Bismuth	3	„
	Determination of Chromium	3	„
	Determination of Cobalt	3	„
	Determination of Copper	3	„
	Determination of Copper Sulphate	3	„
	Determination of Iron	3	„
	Determination of Manganese	3	„
	Determination of Nickel	3	„
	Determination of Phosphate	3	„
	Determination of Vanadium	3	„

Classification	Test	Part	Colour Code
Milk and Milk Products	Determination of Formaldehyde	4	Orange
	Determination of Developed Lactic Acid	7	Dark Blue
	Determination of the Biochemical Oxygen Demand (B.O.D.)	7	„ „
	Colour Grading of Milk and Milk Products	7	„ „
	Determination of Penicillin in Milk	7	„ „
	Quality Grading of Milk for efficiency of pasteurisation	7	„ „
	Determination of Phosphatase	7	„ „
	Quality Grading of Milk (Resazurin test)	7	„ „
Non-Ferrous Metals (See:—Metallurgy)			
Noxious Vapours (See:—Toxic Gasses)			
Oils— Fish & Vegetable	Determination of Vitamin A	4	Orange
	Quality Testing of Fats and Oils for Rancidity	7	Dark Blue
	Quality Grading of Active Carbon	7	„ „
Oils—Lubricating	Determination of Sulphur	3	Brown
	Determination of Cobalt	3	„
Oils—Mineral	Determination of Cobalt	3	„
	Quality Grading of Active Carbon	7	Dark Blue
	Determination of Copper	3	Brown
	Determination of Carbon Disulphide	4	Orange
	Determination of Sulphur	3	Brown
	Determination of Thiophen	4	Orange
Paint	Colour Grading of Shellac and Varnish (Paint Research Station Scale)	7	Dark Blue
	Colour Grading of Varnishes, Oils, Lacquers and Resins (Gardener Scale)	7	„
	Determination of Cobalt	3	Brown
	Determination of Lead	3	„
Paper	Determination of Pentachlorphenate	4	Orange
	Determination of Titanium	3	Brown
	Determination of Dichlorophene	4	Orange
Pesticides (See:—Pest Control)			
Pest Control	Determination of Pentachlororphenates	4	Orange
	Estimation of Humidity	7	Dark Blue
	Determination of D. D. T.	4	Orange
	Determination of Nicotine	4	„
	Determination of Lead	3	Brown
	Determination of Copper	3	„
	Determination of Dichlorophene	4	Orange
Petroleum Products (See:—Oils—Mineral)			

Classification	Test	Part	Colour Code
Pharmaceutical Preparations	Determination of Bismuth	3	Brown
	Determination of Morphine	5	Green
	Determination of Vitamin A	4	Orange
	Determination of Formaldehyde	4	„
	Determination of Silver	3	Brown
Photography	Determination of Silver	3	„
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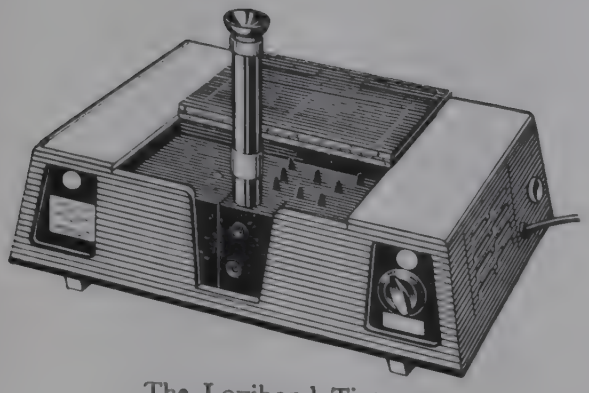
Plastics	Determination of Methyl-α-chloro-acrylate	4	Orange
	Determination of Nonox C.I.	4	„
	Determination of Phenols	4	„
	Determination of Sunconox	4	„
	Colour Grading of Water-white Liquids	7	Dark Blue
	Colour Grading of Varnishes, Oils, Lacquers and Resins	7	„ „
Pathology (See:—Clinical Estimations)			
Pre-Packaging	Estimation of Humidity	7	Dark Blue
Cut Bread	Determination of Chlorine	3	Brown
Potatoes			
Milk			
Printing	Determination of Pentachlorphenates	4	Orange
	Determination of Titanium	3	Brown
	Determination of Silver	3	„
	Determination of Formaldehyde	4	Orange
Public Health	Determination of Ammonia	3	Brown
(See also:—	Determination of Chlorine	3	„
Drainage (Roads)	Determination of Copper	3	„
Effluents	Determination of Formaldehyde	4	Orange
Fumigation	Determination of Lead	3	Brown
Foodstuffs	Determination of Nickel	3	„
Swimming Pools	Determination of Nitrate	3	„
Toxic Gasses)	Determination of Nitrite	3	„
	Determination of Oxygen	3	„
	Determination of Pentachlorphenates	4	Orange
	Determination of Phenols	4	„
Resins (See also:—Plastics)	Colour Grading of Resins (Gardener Scale)	7	Dark Blue
	Determination of Formaldehyde	4	Orange
	Colour Grading of Water-white Liquids (Hazen)	7	Dark Blue
	Colour Grading of Shellac and Varnish (Paint Research Station Scale)	7	„ „

Classification	Test	Part	Colour Code
Road Lines (See:—Paint)			
Rot Proofing	Determination of Copper	3	Brown
	Determination of Pentachlorphenol	4	Orange
Rubber Production	Determination of Copper	3	Brown
(See also:—	Determination of Manganese	3	,,
Soil Fertility)	Determination of Sunconox	4	Orange
	Determination of Nonox	4	,,
Sand	Quality Grading of Sand	7	Dark Blue
Sewage Disposal (See:—Effluents)			
Synthetic Resins, Glues & Sizes	Determination of Formaldehyde	4	Orange
Shellac	Colour Grading of Shellac	7	Dark Blue
	Colour Grading of Varnishes, Oils, Lacquers and Resins	7	,, ,,
	Colour Grading of Water-white Liquids (Hazen)	7	,, ,,
Soft Drinks (See:—Beverages—Non-Alcoholic)			
Soil Analysis	Determination of Carbon Tetrachloride	4	Orange
	Determination of Carotene	4	,,
	Determination of Cobalt	3	Brown
	Determination of Copper	3	,,
	Determination of D.D.T.	4	Orange
	Determination of Iron	3	Brown
	Determination of Magnesium	3	,,
	Determination of Manganese	3	,,
	Determination of Nicotine	4	Orange
	Determination of Nitrate	3	Brown
	Determination of Nickel	3	,,
	Determination of Phosphate	3	,,
	Determination of Pyridine	4	Orange
	Determination of Sodium	3	Brown
	Determination of Sulphate	3	,,
	Determination of Vanadium	3	,,
Sugar Manufacturers (See also:— Boiler Water)	Determination of Noxious Nitrogen	3	,,
	Determination of Sugar	4	Orange
	Quality Grading of Active Carbon	7	Dark Blue
	Colour Grading of Beers, Malt Worts and Caramel Solutions (E.B.C.)	7	,, ,,
Swimming Pools	Determination of Ammonia	3	Brown
	Determination of Bromine	3	,,
	Determination of Chlorine	3	,,

Classification	Test	Part	Colour Code
Swimming Pools—*continued*	Determination of Nitrate	3	Brown
	Determination of Nitrite	3	,,
	Determination of Oxygen	3	,,
	Testing of Swimming Pools	7	Dark Blue
Tar Products (See also:—Oils—Mineral)	Quality Grading of Refined Lower Boiling Products of Coal Tar	7	Dark Blue
	Colour Grading of Refined Cresylic Acid	7	,, ,,
Textiles	Determination of Chlorine	3	Brown
	Determination of Copper	3	,,
	Determination of Dichlorophene	3	,,
	Determination of Formaldehyde	4	Orange
	Determination of Iron	3	Brown
	Determination of Lauryl Pentachlorphenol	4	Orange
	Determination of Sulphate	3	Brown
Tobacco (See also:—Soil Analysis)	Determination of Nicotine	4	Orange
	Estimation of Humidity	7	Dark Blue
Toxic Gasses	Determination of Aniline	6	Pale Blue
	Determination of Arsine	6	,, ,,
	Determination of Benzene	6	,, ,,
	Determination of Carbon Disulphide	6	,, ,,
	Determination of Carbon Tetrachloride	4	Orange
	Determination of Chlorine	3	Brown
	Determination of Chlorinated Hydrocarbons	6	Pale Blue
	Determination of Chloroform	6	,, ,,
	Determination of Hydrogen Cyanide	6	,, ,,
	Determination of Hydrogen Sulphide	6	,, ,,
	Determination of Lead Fumes	6	,, ,,
	Determination of Mercury Vapour	6	,, ,,
	Determination of Methyl α-chloro-acrylate	6	,, ,,
	Determination of Nitrobenzene	6	,, ,,
	Determination of Phosgene	6	,, ,,
	Determination of Sulphur Dioxide	6	,, ,,
	Determination of Styrene	6	,, ,,
	Determination of Toluene	6	,, ,,
Varnish (See:—Paints)			
Vinegar (See:—Foodstuffs)			
Veterinary (See also:—Clinical Estimations)	Determination of Cobalt	3	Brown
	Determination of Magnesium	3	,,

Classification	Test	Part	Colour Code
Water (See:— Boiler Water Drainage (Roads) Drinking Water Effluent Swimming Pools)			
Warehouses (See also:— Pest Control)	Estimation of Humidity	7	Dark Blue
Whisky (See:— Alcoholic Drinks)			

Introduction



The Lovibond Tintometer

THE LOVIBOND SCALE

The Lovibond Colour Scale was devised about 1885 by J. W. Lovibond. It consists of three series of coloured glass slips, one of red (magenta), one of yellow, and one of blue. Each series is a linear scale of the same colour, increasing by equal steps from the palest shade perceptible to a fully saturated (spectral) colour, and is divided into units and decimals of a unit. The colours are permanent and fadeless, and are exactly reproducible by the makers (The Tintometer Ltd., of Salisbury, England). By superimposing suitably chosen glasses, any colour can be matched—there are in all nine million possible combinations of these glasses—and a grey series down to black can also be achieved. All colours can thus be assembled and recorded for future use at will.

The Lovibond Comparator

Lovibond units of colour have received international recognition, and standardising bodies in over 20 different countries quote colours in Lovibond nomenclature.

The Lovibond Scale has also been related to the Commission Internationale de l'Eclairage system of colour specification (R. K. Schofield, *J. Sci. Inst.*, 1939, **16**, 74) so that records are interchangeable between the two systems; and a specification expressed in C.I.E. trichromatic co-ordinates can be converted into Lovibond units, assembled, and looked at as an actual sample of the colour.

The Special-Purposes Lovibond Comparator

The Lovibond Tintometer contains a selection of colour slides whereby any colour can be assembled at will, while the Lovibond Comparators and the B.D.H. Lovibond Nessleriser contain actual glass colour standards prepared from composites of Lovibond glasses to match the particular colours produced in any specific test. The disc method is quicker and simpler to use for routine tests, but the Tintometer enables original work to be carried out, because every possible colour may be matched at will.

The B.D.H. Lovibond Nessleriser

This book contains details of many chemical tests which are in current use, and which have been specially developed for use with Lovibond standards in co-operation with many leading laboratories, but new tests are continually being added, and the list of possibilities may be considered infinite.

This sixth edition of "Colorimetric Chemical Analytical Methods" has been produced as a result of requests from chemists all over the world. The opportunity has been taken to bring the text completely up-to-date at the time of writing and, as new tests are continually being added to the list and the procedures of old tests revised, the book has been designed in loose-leaf form so that constant revision can be carried out with the minimum of inconvenience. Enquiries concerning other colorimetric tests are welcomed by The Tintometer Ltd.

The published price includes a free service for two years of revision leaflets, which will be issued from time to time to those purchasers of the book who despatch the accompanying addressed postcard.

Quantitative colorimetric chemical analysis occupies a large place in the work of the chemist and he continually seeks more specific and more sensitive tests.

Such tests are widely used for determining minute quantities of known radicles, but are not, of course, used in determining major constituents where they constitute a large percentage of the whole. In such cases, gravimetric or volumetric methods are employed rather than colorimetric.

Although, in its literal sense, the term colorimetry denotes the measurement of colour, the tests described in this volume do not in general involve the *measurement* of colour, but rather the comparison of a colour against permanent glass colour standards. The tests described are of two types :—

a. colour grading to a specification

b. chemical tests in which the presence of a certain radicle is proved by the development of a specific colour by means of a chemical reagent, and the amount of that radicle is assessed by means of the intensity of the colour produced.

The procedures in this book have been checked and revised by workers in many fields, and they thus represent the combined experience and skill of a very large number of leading analysts working in some of the foremost laboratories of the world.

Basically, the procedure behind quantitative colorimetric chemical analysis is to produce a coloured solution from the sample by means of a closely defined chemical procedure, and to compare this colour against similarly prepared comparison standards of known assay. The preparation of these standards, however, requires great skill and care and is very time consuming.

Lovibond glass colour standards in the apparatus described replace this difficult procedure. These standards are available as an exact match of standard solutions carefully prepared by skilled workers in the laboratories of specialists, in different parts of the world, who co-operate with the Tintometer Limited. They are then checked and cross-checked in other laboratories. Being absolutely permanent and fadeless they can be relied upon indefinitely and thus not only save time, but simplify procedure and give confidence. Any error in carrying out the test can be detected at once, because the test solution then appears the wrong colour and the operator is thus warned to check back on the reagents and technique.

The book has been primarily designed for use with visual methods and the notes in the following section describe the various instruments referred to in the book.

Part 1

Lovibond Apparatus

Lovibond Permanent Glass Standards in Lovibond Equipment

for performing the tests described in this book

The Lovibond Comparator

THE pocket size Lovibond Comparator consists of a moulded case (Fig. 1) furnished at the back with an opal glass screen, and with two compartments to receive test tubes or rectangular cells containing the liquid under examination. In the front portion are two circular holes, situated side by side opposite the opal screen and coinciding with the vessels containing the solutions under examination.

A moulded disc, fitted with up to nine glass colour standards, fits into the recess in the lid of the comparator and can be rotated on the moulded centre hub. The rim of the disc is inserted under a fixed spring clip at the bottom corner of the comparator lid and then the disc slips easily into place over the centre hub where it is held by moving the sliding spring clip at the top corner of the comparator lid. Each colour standard in turn passes in front of the left-hand cell compartment when the disc is rotated inside the closed comparator. As the disc is rotated, the value of the colour standard visible in the left-hand aperture appears at the indicator recess near the bottom right-hand corner of the comparator case.

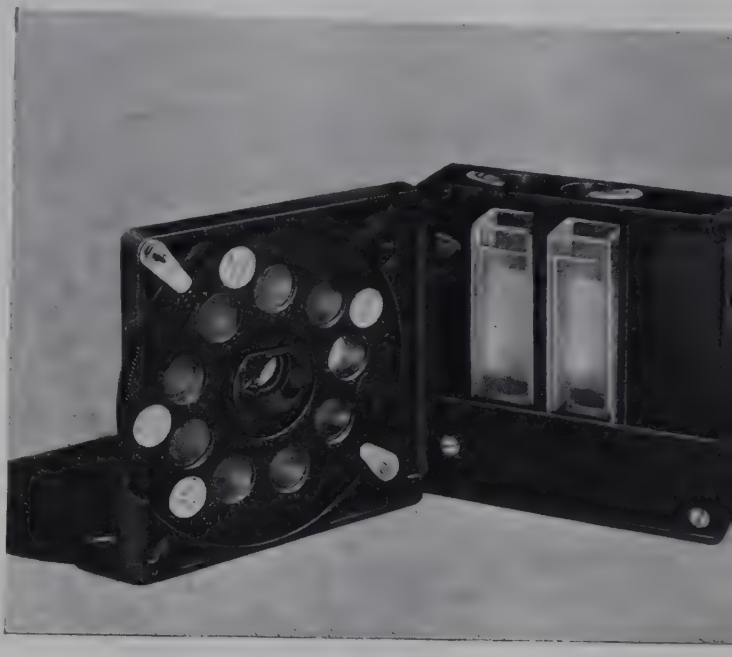


Fig. 1 **The Lovibond Comparator**

Fig. 2 **The Lovibond Comparator in use, being held up to a source of diffuse daylight. A north window is best.**

Discs are available for use in carrying out numerous quantitative chemical and clinical tests. Most of the tests for which the comparator is used depend upon (a) the change in colour which takes place, or (b) the intensity of the colour developed, when a measured quantity of an indicator solution or reagent is added to a measured quantity of the test sample. The initial colour of the test sample naturally affects the colour developed, so provision is made for a "blank" or untreated sample of the test solution to be placed behind the colour standard when necessary, in a vessel of the same internal diameter as that containing the test sample to which the indicator or reagent has been added. This arrangement automatically compensates the colour standard for any inherent colour in the solution under test and thus ensures that the same colour standards are applicable for use with either coloured, slightly turbid, or water-clear solutions.

For use in the grading of certain products, discs containing sets of arbitrary colour standards are available. Also, special discs may be prepared in which the colour standards match a series of coloured samples supplied by an individual customer.

Method of Use

The test solution, when ready for comparison with the colour standards, is poured into the appropriate vessel* and placed in the right-hand compartment; the case is closed and the disc is revolved until the colour standard most nearly matching the sample appears in the left-hand aperture. The value of the colour standard, visible at the indicator recess, is then recorded. The glass colour standards with which the moulded discs are fitted are permanent in hue, saturation and brightness.

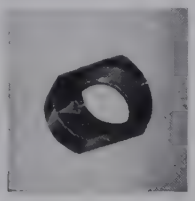


Fig. 3
Dulling Screen

In a few tests the colour developed is brighter than the Lovibond glasses of corresponding hue. For these a "dulling" screen must be used, each screen being appropriate to one particular test only. A "dulling" screen consists of a glass colour standard fitted into a moulded holder which slides into the recess in the centre hub in the lid of the comparator. See Fig. 3. (There is a small "finder" arrow on the screen moulding which indicates the way in which it should be inserted). The screen then comes in front of the test solution and, being of complementary hue, reduces its brightness to that of the disc standards. For most tests **no** screen is required.

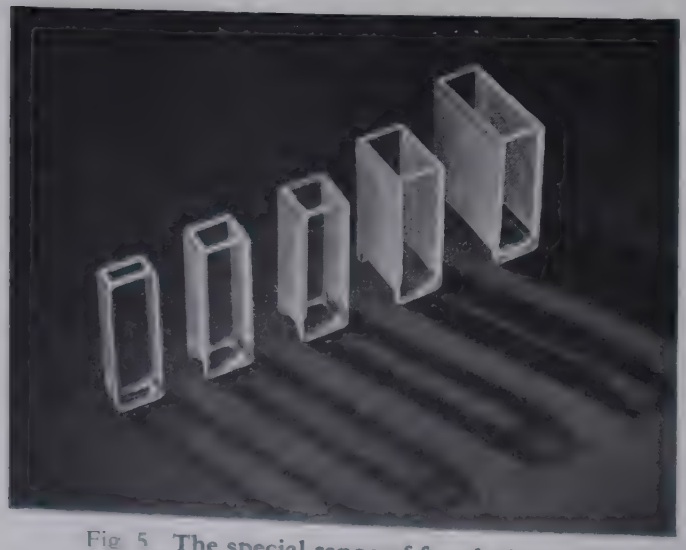


Fig. 5 **The special range of fused glass cells for the S.P. Lovibond Comparator**

*The standard Lovibond test tubes are graduated at 50ml and have an external diameter of 13·5mm and a thickness of glass of approx. 0·2mm. For [illegible] brand fused glass cells, produced to work with optically plane faces and an internal optical path of 13·5mm, is recommended.

Fig. 4 **The Speical-Purposes Lovibond Comparator**

The Special-Purposes Lovibond Comparator

The Special-Purposes Comparator (Fig. 4) has been designed to deal with those tests which require a cell of greater optical depth than can be accommodated in the Lovibond Comparator. While the Special-Purposes Comparator can be used for all tests normally carried out with 13.5 mm. cells in the Lovibond Comparator, its size makes it less convenient to handle. Its purchase is therefore only recommended when some of the tests to be carried out require the use of larger cells.

The front of the instrument, with the provision for the disc and screen, is exactly the same as that of the smaller Lovibond Comparator. The top of the Special-Purposes Comparator is hinged to give access to longer cell compartments designed to accommodate cells having optical depths of up to 4 cm. (The special range of optically accurate fused glass cells is shown in fig. 5).

The back of this comparator is hinged and is fitted with a white opal glass diffusing screen, held in place by a spring clip, for normal use. If opaque samples are to be tested the diffusing screen is replaced by two opal glass reflectors. The back is then fixed half-open to provide a white background when the instrument is held horizontally. (Fig. 6).

Fig. 6

Method of Use for Liquids

When the top lid is opened, compartments are visible for holding cells or test tubes. For tests requiring the liquid to be placed in test tubes, slide the inner frame into position so that the two circular holes come immediately adjacent to the face of the comparator, remote from the diffusing screen, and above the two holes sunk in the base block. The test tube containing the prepared test solution is then placed in the hole behind the centre aperture in the face, and if a "blank" solution is used, this is placed in the hole behind the left-hand aperture so that the colour of the "blank" is superimposed on the glass colour standard in the disc. The top and hinged back are then closed, the appropriate disc placed in the front of the comparator, and the colour matched. It is essential to use the standard 13.5 mm. colourless test tubes supplied by the makers of the comparator, as all colours are standardised on this exact diameter tube.

If the liquid is to be examined in a rectangular cell of 13.5 mm. the inner frame is reversed, so that the round holes are on the other side, and space is available next to the front face for the cell, and a "blank" if required (see Fig. 4). When using other sized cells, this inner frame is removed entirely, and a special wooden block is inserted to hold the cells firm. This is fitted inside the comparator so that the two tongues fit into the grooves at the sides of the instrument, and the narrow channel registers over the raised ridge in the bottom of the comparator. As when using test tubes, the top is shut to exclude extraneous light while examining the colour of the liquid.

For certain tests, the Lovibond Comparators are used on a stand with the diffusing screen removed so that the user can look right through the comparator on to the sample below. Examples are the Resazurin and other milk tests, (see Fig. 7) and the test for humidity (see Fig. 8).

Fig. 7

Fig. 8

The Correct Source of Light

In no case should observations be made in direct sunlight: north or diffused daylight gives the best conditions.

Special equipment (see Figs. 9 and 10) has been designed for use in a laboratory which lacks good north daylight or where readings must be taken at night.

Reference to "North daylight" throughout this book infers northern hemisphere; when used in the southern hemisphere, the necessary alteration should be understood.

Notes

All tests for which the Lovibond Pocket Comparator is available may be carried out on the Special-Purposes Comparator and the same discs are interchangeable. For the direct measurement of Haemoglobin (Part 5), however, a special interior fitting must be added.

As stated above, for accurate work it is strongly recommended that rectangular cells are used in the place of round test tubes. These cells have the same optical depth (13.5 mm.) as the standard test tubes. Identical depths of colour are therefore produced, and the same standard colour discs apply. The rectangular cells, having a uniform optical depth across the field of view, produce a more uniform colour and facilitate matching.

Convenient carrying cases to hold a Lovibond Comparator and a selection of discs and bottles can be supplied to order.

Daylight Equipment for Round-the-clock Use

for Lovibond Comparators

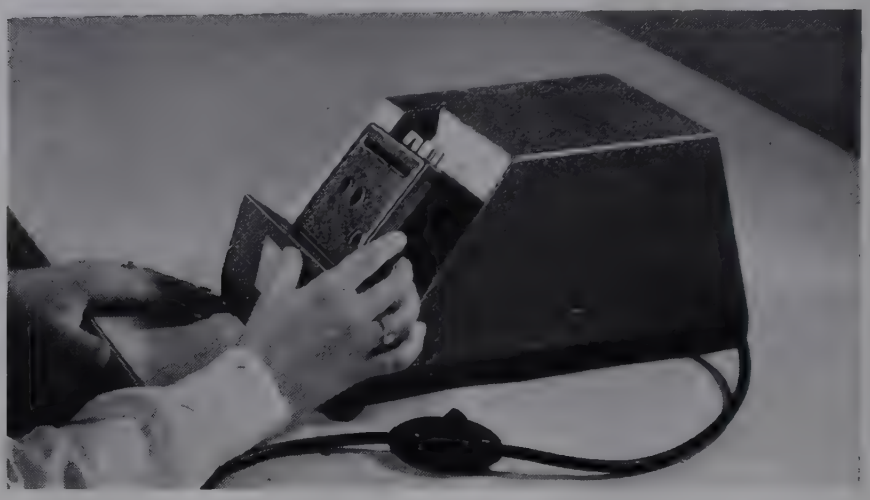


Fig. 9 **(Universal Model AF218F)**

For those occasions when a good diffused daylight is not available to use with a Lovibond Comparator, the above cabinet provides a convenient alternative source of corrected white light.

Where there are large voltage fluctuations on the mains supply, or where results are required for referee work, cabinet Model AF218A may be preferred.

Fig. 10 **Arbitration Model AF218A**

This substitute for natural daylight is strongly recommended for those cases where exact reproducibility of illumination is essential (e.g. when working to very fine limits, or when referee work is to be carried out) and for use in laboratories where the mains electricity supply is subject to large fluctuations in voltage. It is essential that the particular type of lamp chosen, and the specially developed filter, should always be used together to produce the correct illumination, which is the result of much experimental work.

Available only for A.C. current. The standard equipment, Model A, is for 200/240 volts, 50 cycles, Model B is for 100/120 volts, but models for other voltages can be made to order.

Fig. 11

Fig. 12

The B.D.H. Lovibond Nessleriser

This instrument makes use of the same principle as the Lovibond Comparator; it has discs of 9 glass colour standards and uses the method of compensation for the inherent colour of the original solution. Instead of viewing across the width of a test-tube or small cell, however, Nessler tubes are used and the operator looks down through the depth of the tube, thus allowing very pale colours to be examined and small steps in concentration distinguished. For example, a chlorine concentration of as low as 0.01 parts per million develops a colour which can be seen in this depth, and therefore this instrument is valuable for the chemical estimation of very low concentration of certain radicles.

A special light source is also available for use with the Lovibond Nessleriser in circumstances where adequate north daylight is not available.

ILLUSTRATION OF THE GLASSES OF

THE LOVIBOND COLOUR SCALE

These transparent glass colour standards are viewed against a white background, and can be used singly, or combined together as pairs. or as three glasses (one of each colour).

How a special Lovibond disc of permanent fadeless glass colour standards is prepared

The discs used in Lovibond Comparators are prepared by the makers from combinations of standard glasses from the Lovibond Scale. The range of coloured solutions required for a particular test is prepared (Fig. 13) and each colour is matched, in a standard cell, by a suitable combination of Lovibond glasses. The appropriate glasses are then cemented together, to form a permanent fadeless standard, and mounted in a disc.

Once the colour formula has been determined in this manner, identical standards can be reproduced at any future time and in any quantity. The method of colour measurement and reproduction is so accurate that any colours can be reproduced in the form of permanent standards. Discs can thus readily be produced to the requirements of individual users, either for use in the colour grading of particular products, or for use as standards in special colorimetric analyses. Once the colours have been measured and a prototype disc approved, the cost of producing copies is no greater than that of producing a standard disc.

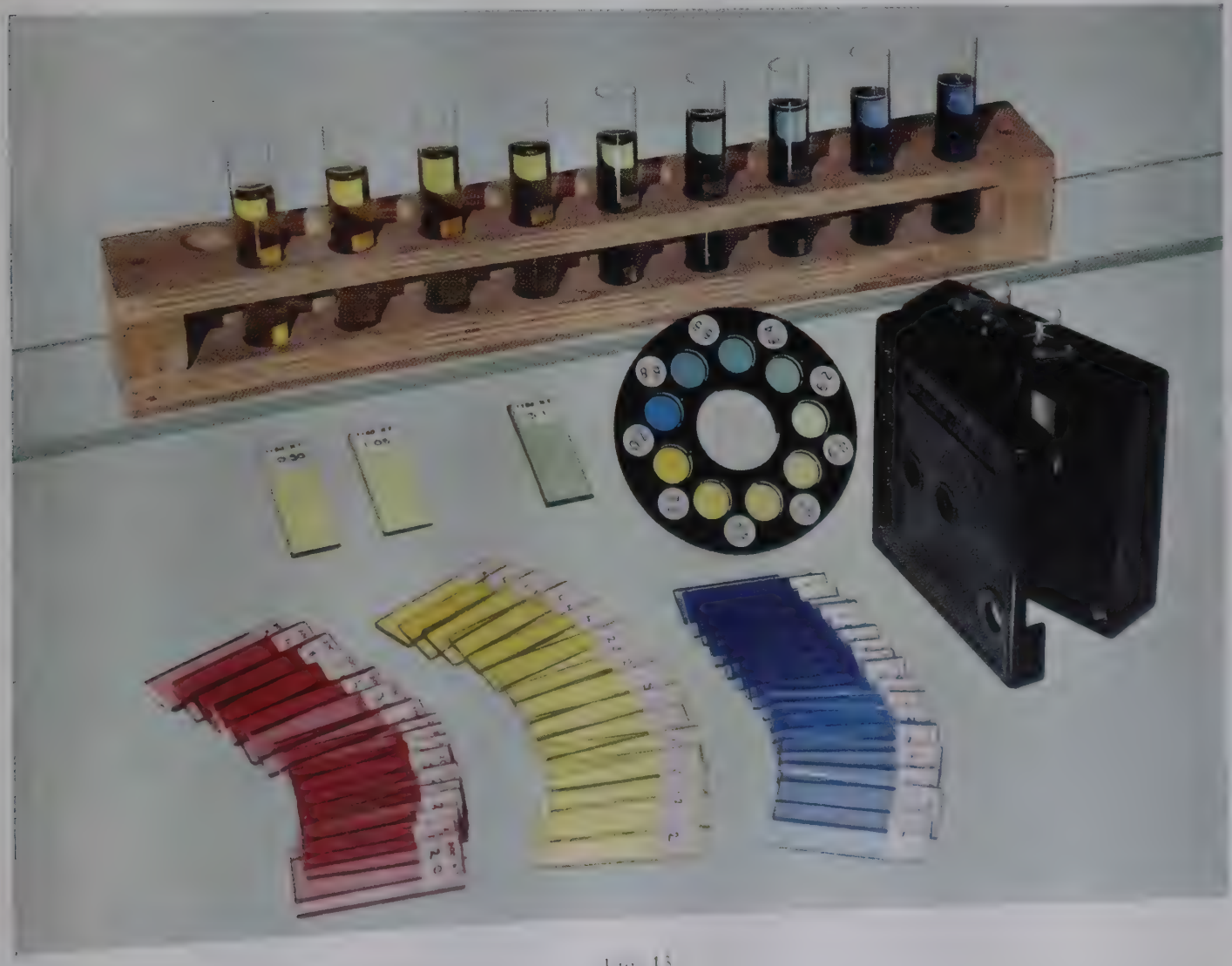


Fig. 13

The Lovibond Tintometer

The Lovibond Tintometer consists essentially of an apparatus to make correct use of the Lovibond scale of permanent glass colour filters. There are three separate Lovibond scales, one for each of the three subtractive primary colours, red, yellow and blue. Each scale consists of over 200 accurately graded steps, forming an additive scale, such that e.g. 3.0 is exactly the same colour as three 1·0 units. If glasses of two different colours are superimposed, the secondary colour is obtained—thus, red and yellow together produce orange, yellow and blue produce green, and red and blue produce violet. Equal values of all three colours together produce neutral grey. *Any* colour can thus be expressed as being matched by such and such a combination of Lovibond glasses. For example, a strong orange colour might be recorded as 10 units of Red and 15 of Yellow. Suitable selections of glasses are fitted into moulded plastic racks in the B.D.H. pattern Tintometer (Fig. 14), and these can be moved along in grooves in the instrument. Thus any desired combination can be produced at will in the field of view by the operator, and the values of the glasses used are recorded as the colour. There are many thousand of these instruments in use throughout the world and the results obtained on one can be duplicated on any other.

To provide a standard illumination and a means of holding the samples, a White Light Cabinet (Fig. 15) is provided. Solid samples are viewed by reflected light, and liquids are placed in accurately graded glass cells and viewed by transmitted light against a standard white background.

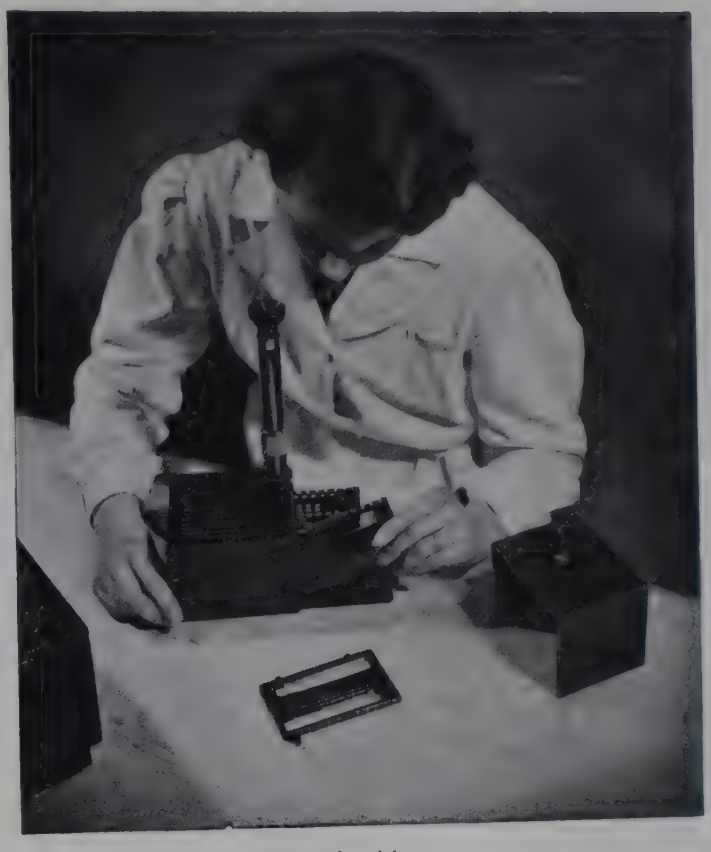


Fig. 14

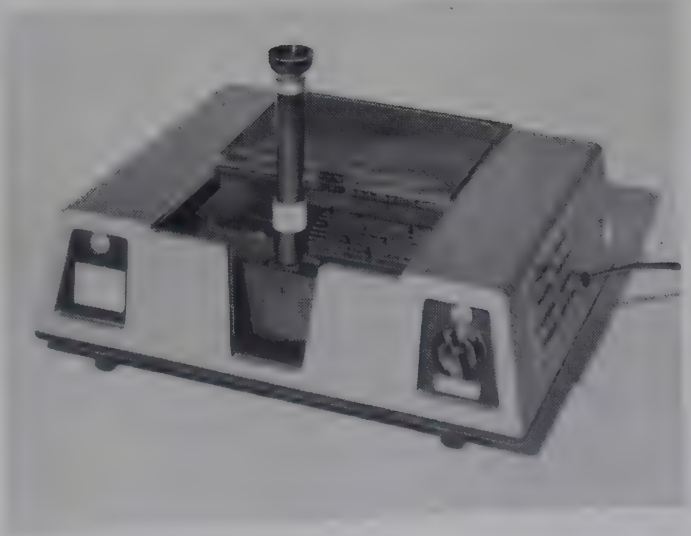


Fig. 15

Part 2

The Colorimetric Determination of *p*H

The Determination of *p*H Values (1)

Introduction

In nearly all branches of research and industrial work, from sugar manufacture to sewage disposal, from brewing to boiler-water control, it has long been realised that the control of the acidity and alkalinity of the various solutions used is of paramount importance, since this factor frequently has a marked effect on yield, quality, stability, etc.

Explanation of *p*H*

The essential point to be realised is the difference between **total acidity** and **active acidity (or alkalinity)**. The total acidity is measured by the usual titration method, and furnishes the answer as to **how much** of the given acid there is in a given volume of the solution: but this is no guide as to the **intensity** of the acidity, which, of course, is the controlling factor in many processes. For instance, equivalent quantities of hydrochloric acid and boric acid in solution show exactly the same acidity by titration, but there is clearly a large difference in the acidic properties of these solutions: this difference one may call the **intensity** of acidity. In the past, failure to appreciate this distinction, and attempts made to control processes by rough test-papers or by titration, have resulted in failures and wastage in chemicals and time.

Of recent years this matter has been fully investigated and shown to be dependent on the Hydrogen Ion Concentration. According to the theory of electrolytic dissociation, all liquids of which water is a constituent contain free, positively charged, hydrogen ions, and negatively charged hydroxyl ions. When the numbers of these two ions present in a liquid are equal, the liquid is said to be neutral. Thus, a litre of pure freshly-distilled water contains one ten-millionth of a gram (10^{-7}) of ionised hydrogen and an equivalent amount of hydroxyl ion, and the water is neutral. Addition of an acid to water increases the concentration of the hydrogen ions and decreases the concentration of the hydroxyl ions: the water becomes acid in reaction, and, according to the increase in the hydrogen ion concentration, so the **active acidity** increases. The converse effect is obtained by the addition of an alkali to water.

The product of the concentration of these two ions expressed in grams is **constant at a given temperature,** so that if one is known the other is easily calculated, and it is usual to speak only of the Hydrogen Ion Concentration. This concentration is expressed as grams of active or ionised hydrogen per litre of the liquid. Decinormal hydrochloric acid is almost ionised, and 1 litre contains approximately 0.1 gram ionised hydrogen, and the H ion concentration is 0.1 or 10^{-1}. At the other extreme, decinormal sodium hydroxide contains 0.000,000,000,000,086 gram of ionised hydrogen per litre, and its H ion concentration is expressed at 8.6×10^{-14} or $10^{-13.07}$. This method of expression is clumsy, so the term "*p*H" has been adopted, the letter "*p*" standing for the German "potenz" or power (mathematical). **The *p*H value is the logarithm to the base 10 of the reciprocal of the hydrogen ion concentration,** i.e., the index of the hydrogen ion concentration with the negative sign changed to positive. Thus in the examples given above, the *p*H of decinormal hydrochloric acid is 1.0, of decinormal sodium hydroxide is 13.07, and of pure freshly-distilled water 7.0.

Thus simply, a solution having a *p*H value of 7 is neutral (neither acid or alkaline), and as this value decreases below 7 the hydrogen ion concentration increases, and with it the active acidity. Above 7, the reverse is the case, the hydroxyl ion concentration increasing, and with it the alkalinity. It must be remembered that this is a logarithmic scale, so that *p*H 5 represents ten times more ionised hydrogen than *p*H 6, and *p*H 4 one hundred times more ionised hydrogen than *p*H 6, and so on.

*British Standard 1647: 1950 "*p*H Scale" has adopted an operational standard, and defined a primary standard in terms of chemical constitution. It then defines the difference in *p*H between any two solutions in terms of electromotive force. The above paragraph, however, may still be taken as an adequate explantion of *p*H for all ordinary purposes. For a detailed treatment of this subject readers are recommended to consult "The Book of *p*H" by R. B. Webber, M.A. (Tintometer Ltd., Salisbury, 1957, 30/-).

Indicators

In measuring *p*H values, advantage is taken of the fact that certain dyes, known as indicators, change their colour in definite and reproducible manner and degree, according to the *p*H value of the solution with which they are mixed. The simplest and best known of these is litmus, which is red in an acid solution and blue in an alkaline solution. As, however, it has a very wide range between its extreme acid colour (*p*H 4.6) and its extreme alkaline colour (*p*H 8.4) and as the actual colours vary with different qualities of litmus, it is unsuitable for the determination of *p*H values. To ascertain, in addition to the fact that a solution is acid, precisely what degree of acidity it possesses, recourse is had to indicators which show a complete colour change through a short *p*H range. Thus bromo-thymol blue shows a full yellow colour in acid at *p*H 6.0 and as the *p*H value increases the colour changes gradually to green at neutral and then to blue as the solution becomes alkaline, so that at *p*H 7.6 it is deep blue. Other indicators show their full colour change all on one side or the other of neutral; for example, cresol red is yellow at *p*H 7.2 (slightly alkaline) and changes through orange to purplish red at *p*H 8.8 (more highly alkaline). These colour changes are constant for given concentrations of each indicator. It is only necessary therefore to have sufficient indicators available, spanning the *p*H scale, and to find some means of recording the colour changes, to be able to ascertain the *p*H value of any aqueous liquid.

Principle of the method

The colour changes of indicators are most easily identified with the Lovibond Comparator, with which discs are supplied fitted with the Lovibond permanent glass colour standards. These show in nine steps the colour changes undergone by any given indicator when added to colourless liquids of the various *p*H values within its range. Thus, to revert to the example of bromo-thymol blue, if a stated quantity of this indicator solution be added to a liquid whose *p*H value is between 6.0 and 7.6, the colour produced will be matched by one of the steps on the disc, and the numerical value noted. Of course, if the value were below *p*H 6.0 or above *p*H 7.6, the colour would only go as far as the end value and remain there, and read for example, *p*H 6.0 when the true value might be 5.0 or even lower. In such a case, another indicator should be employed whose range is lower on the acid scale. Some indicators have a double range of usefulness. For example, cresol red changes from red to yellow as the *p*H value drops from 8.8 to 7.2, remains at this colour until the *p*H value has dropped as low as *p*H 1.8, and then commences to reverse the process as the *p*H value drops still lower in the acid direction, regaining a red colour at *p*H 0.2. There are thus two discs for this indicator. A sufficient number of indicators and discs are available to cover the whole range from *p*H 0.2 to 14.0 in small steps, with an overlap of readings between each adjacent pair, so that any reading at the end-point of an indicator should be confirmed by repeating the test with the next indicator. If one is dealing with a solution whose *p*H value is totally unknown, recourse should be had to the B.D.H. Universal Indicator, which has the enormous range of *p*H 4.0—11.0, changing through all the colours of the spectrum in correct order. This, of course, gives a very approximate answer, which is only intended to serve as a guide to which indicator is appropriate for accurate determination.

In the case of certain indicators, the colour developed is brighter than the corresponding Lovibond glass. In these instances a "dulling" screen must be used in conjunction with the disc. This is a glass of a complementary colour, and is superimposed over the test solution to reduce the two fields to the same brightness level.

Reagents and Standard Lovibond Comparator Discs

Discs containing coloured glasses standardised on the following B.D.H. Indicators are available, showing colour changes in steps of 0.2 except where otherwise stated :—

*p*H range	Indicator	Standard Disc	Notes
0.2 — 1.8	Cresol Red	2/1Y*	
1.0 — 2.6	*m*-Cresol Purple	2/1W	
1.2 — 2.8	Tropæolin 00	2/1I	
1.2 — 2.8	Thymol Blue †	2/1A	
2.8 — 4.4	Bromo Phenol Blue	2/1B	

*p*H range	Indicator	Standard Disc	Notes
3.0 — 4.6	B.D.H. 3046	2/1V	
3.6 — 5.2	Bromo Cresol Green†	2/1C	
4.0 — 8.0	B.D.H. 4080	2/1CC	in steps of 0.5
4.4 — 6.0	B.D.H. 4460	2/1D	
4.4 — 6.0	Methyl Red	2/1E*	
4.8 — 6.4	Chloro Phenol Red	2/1F	
5.2 — 6.8	Bromo Cresol Purple	2/1G	
6.0 — 7.6	Bromo Thymol Blue†	2/1H	
6.8 — 8.4	Phenol Red†	2/1J*	
7.0 — 8.6	Diphenol Purple†	2/1O	
7.2 — 8.8	Cresol Red	2/1K	
7.6 — 9.2	*m*-Cresol Purple	2/1Z*	
8.0 — 9.6	Thymol Blue†	2/1L	
8.6 —10.0	B.D.H. 8610	2/1U	8 standards only
9.0 —11.0	B.D.H. 9011	2/1M	5 standards only
10.0 —14.0	B.D.H. 1014	2/1BB	in steps of 0.5
4.0 — 8.0	B.D.H. Soil	2/1N	in steps of 0.5
4.0 —11.0	B.D.H. Universal	2/1P	in steps of 1.0
2.8 — 4.4	2 : 4 dinitrophenol α	KAR	*p*H of glue and gelatine
4.0 — 5.6	2 : 5 dinitrophenol γ	KAS	monochromatic indicator
			ditto
5.4 — 7.0	*para* nitrophenol	KAX	ditto
6.8 — 8.4	*meta* nitrophenol	KAZ	ditto
4.8 — 6.4	Ethyl Red	2/1AA*	for the *p*H of formaldehyde solutions in the plastics industry

*These discs must be used in conjunction with a dulling screen.

†These indicators are available in tablet form.

Indicators made by British Drug Houses Ltd. are specially prepared to conform to the colours on the Lovibond discs.

Technique

Fill both test tubes, or cells, to the 10 ml. mark with the solution to be tested, and to the right-hand tube, only, add the appropriate quantity of B.D.H. indicator with the pipette provided. Use 0.5 ml. indicator, except only in the case of B.D.H. Universal Indicator, when only 0.1 ml. is used (0.2 ml. if the solution proves to be acid). Do not immerse the tip of the pipette beneath the surface of the liquid being tested. Carefully mix indicator and liquid, by stirring with a clean glass rod, or by pouring from one tube to another. Insert the appropriate disc in the recess in the lid, revolve until the nearest match is obtained, and read the *p*H value in the indicator recess at the bottom right-hand corner.

If there is any doubt as to which is the appropriate indicator to use, carry out the test first with the B.D.H. Universal Indicator to obtain an approximate reading, then select the indicator with the mid-point nearest this approximate determination.

Steps on most of the discs are of 0.2 *p*H divisions, and values can usually be interpolated between these. Carefully wash test tubes and pipette after use. The reason for using a "blank" in the left-hand test tube is to give compensation for any inherent colour or turbidity in the liquid under test. If, for instance, a brownish liquid were being tested, the colour resulting from the mixture of liquid and indicator would be influenced by the inherent colour of the sample, and so would the colour as seen through the glass in the left-hand aperture, and the departure from normal would thus be compensated.

Adjustment of the *p*H value of solutions

The converse of determination of *p*H, i.e., the adjustment of a solution to any required *p*H value, is an easy matter with the Lovibond Comparator. For this work, a Comparator test-tube of the same diameter as the standard tube, but of greater length, can be supplied.

Place in the tube 10 ml. of the solution which is to be adjusted, add 0.5 ml. of the indicator

which covers the required *p*H value, set the disc at the required reading and titrate from a burette with standard acid or alkali, as the case may be, until the appropriate colour is obtained.

The solution must be well mixed between additions from the burette. Note the amount required, and carry out the adjustment on a suitable volume of the solution without adding any indicator.

Determination of *p*H of Neutral Solutions—*p*H 7.0

One of the most difficult *p*H determination is that of distilled water, or water approaching that state of purity. The reason for this difficulty is the lack of " buffering " action in such water. In most liquids one has to test, there are traces of a number of substances or impurities, and the chances are very high that there are present some salts of a weak acid or weak base. The salts act as " buffers " — that is, they strongly resist any change in the *p*H of the solution, and consequently slow down the alteration in *p*H value which occurs when an acid or alkali is added to the solution. For example, if 0.1 ml. of decinormal hydrochloric acid be added to 10 ml. of distilled water with a *p*H or 7.0, the *p*H will be changed to 3.0 ; if however, 0.1 ml. of decinormal hydrochloric acid be added to 10 ml. of decimolar ammonium acetate solution also with a *p*H of 7.0, only a small change in *p*H occurs, and it will require the addition of over 10 ml. of the acid to reduce the *p*H to 3.0. Thus the ammonium acetate solution has exerted a strong buffer action.

Now, indicator solutions are adjusted to the mid-point of their *p*H range, so that they may be either acid or alkaline, according to which indicator is being used. Owing, however, to this " buffering " action which most solutions possess, the *p*H value of the indicator itself does not appreciably affect the result. When, however, **unbuffered** waters are to be tested (e.g., freshly distilled or certain natural waters) the result obtained will be largely influenced by the indicator itself. While there are certain chemical precautions which can be taken to obviate this difficulty, they are not simple, and a much simpler method is available through the use of the B.D.H. Lovibond Nessleriser. In this case, by a suitable re-adjustment of the proportions of test liquid and indicator, the possibility of such errors has been reduced to a minimum.

Notes

1. **"Salt Errors"** It must be understood that the accuracy of *p*H determinations by means of **any** colorimetric method may be affected by " salt " or " protein " errors, and due correction may have to be made. Salt errors are not likely to occur in concentrations below deci Molar. The discs are standardised against " buffer " solutions.

2. **Electrical *p*H meters.** The Lovibond Comparator is invaluable in checking the buffer solutions which are used to standardise *p*H meters.

3. **Advantages of the Comparator.** Compared with other methods of measuring *p*H values, the Lovibond Comparator has the great advantages of extreme simplicity, permanence and strength, and (as compared with electrometric methods) cheapness. An unskilled worker can obtain reliable results without training ; such results are consistent over long periods. The colour glasses are permanent and therefore do not require renewing as is the case with tubes of coloured solutions. The comparator is robust in construction and, complete with disc and the necessary accessories for one indicator, fits into the pocket. By the use of sufficient discs, practically the whole range of *p*H values is adequately covered. Maintenance is negligible, which justifies the initial outlay.

4. For details of complete kits designed for *p*H tests, apply to Tintometer Ltd. Salisbury.

The Determination of *p*H Values (2)

Nessleriser method

Introduction

By using Nessleriser glasses instead of test-tubes for *p*H tests, and looking down through the column of liquid instead of across it, the proportion of indicator solution needed in the liquid is reduced to a minimum. This avoids the considerable errors the reaction of the indicator solution may otherwise introduce into colorimetric determinations of the *p*H of natural waters and other relatively unbuffered solutions.

Principle of the method

Except in the disc for the B.D.H. Universal Indicator the colour glasses of the Nessleriser series of *p*H discs are standardised against the colours produced by adding 0.2 ml. of the respective indicator solution to 50 ml. quantities of standard buffer solutions. Colours in the Universal Indicator disc are standardised on 25 ml. quantities of solution containing 0.1 ml. of indicator in acid solutions and 0.05 ml. in alkaline solutions. When 0.05 ml. cannot be measured conveniently, 50 ml. of solution containing 0.1 ml. of indicator should be prepared and 25 ml. of the mixture used for the test. With the Universal Indicator disc the quantity of solution without indicator used as a blank in the left-hand Nessleriser glass should also be 25 instead of 50 ml.

Reagents and Standard B.D.H. Lovibond Nessleriser Discs

The discs designed for use with the B.D.H. Lovibond Nessleriser are distinguished from those used in conjunction with the Lovibond Comparators by the word "**Nessleriser**" on the moulding. The following discs are available :—

*p*H range	Indicator	Standard Disc	Notes
1.2 — 2.8	Thymol Blue	NLM	
2.8 — 4.4	Bromo Phenol Blue	NLN	
3.0 — 4.6	B.D.H. 3046	NLR	
3.6 — 5.2	Bromo Cresol Green	NLA	
4.0 — 8.0	B.D.H. 4080	NLT	
4.4 — 6.0	Methyl Red	NLL	
4.4 — 6.0	B.D.H. 4460	NLO	
5.2 — 6.8	Bromo Cresol Purple	NLB	
6.0 — 7.6	Bromo Thymol Blue	NLC	
6.8 — 8.4	Phenol Red	NLD	
7.0 — 8.6	Diphenol Purple	NLP	
7.2 — 8.8	Cresol Red	NLE	
7.6 — 9.2	m-Cresol Purple	NLK	
8.0 — 9.6	Thymol Blue	NLF	
9.0 —11.0	B.D.H. 9011	NLG	5 standards only
10.0 —14.0	B.D.H. 1014	NLS	
4.0 —11.0	B.D.H. Universal	NLH	

Technique

Fill one of the Nessleriser glasses to the 50 ml. mark with the fluid under examination and place in the left-hand compartment of the Nessleriser. Place the appropriate quantity of indicator in the other Nessleriser glass and fill to the 50 ml. mark with the fluid under examination, mix thoroughly and place in the right-hand compartment. Stand the Nessleriser before a uniform source of white light — a north window is the best — and compare the colour produced in the test solution with the colours in the standard disc, rotating the disc until a colour match is obtained.

The markings on the discs represent the *p*H values corresponding with the colours in the field of view.

Notes

1. It is important that the liquid being tested should be as clear as possible, as turbidity reduces the amount of light transmitted and consequently dulls down the colours, and that the correct quantities of indicators should be used — see introduction overleaf.

2. B.D.H. Lovibond Nessleriser discs are standardized on a depth of liquid of 113 ± 3 mm. The height, measured internally, of the 50 ml. calibration mark on Nessleriser glasses used with the instrument must be within the same limits. Tests with Nessleriser glasses not conforming to this specification will give inaccurate results.

3. No reliance should be placed on a result indicated by the colours at the extreme ends of the range of an indicator as a *p*H value outside the range will give only an end colour. In such cases, the test should be repeated using an indicator with an over-lapping range.

4. Indicators made by British Drug Houses Ltd., are specially prepared to conform to the colours on the B.D.H. Lovibond Nessleriser discs.

The Determination of the *p*H of Blood

Introduction

The following description has been taken from Hawk and Bergeim[1], by permission. For a complete account of the theoretical considerations regarding acid-base equilibrium and its clinical manifestations, the original text should be consulted.

" Disturbances of the acid-base equilibrium are divided into two clinical types by Peters and Van Slyke[2], viz. the metabolic type, in which the primary disturbance is in the relation between alkalis and acids other than carbonic, and the respiratory type in which the primary disturbance involves the carbon dioxide content of the blood. In the table herewith, the more important conditions associated with the disturbance of the acid-base balance are given, together with their causes and the physiological mechanisms brought into play for their compensation.....It is the consensus of the best opinion at the present time that cases of disturbed acid-base balance can best be diagnosed and their course followed.....by the determination of certain factors which are more or less typical of " acidosis." These include the following :—

1. The alkali reserve of the blood
2. The alkali tolerance of the patient
3. The carbon dioxide tension of the alveolar air
4. The hydrogen ion concentration (*p*H) of the blood

The normal *p*H range of blood is between 7·30 and 7·50. The extreme limits of pathological variation which have been observed are 6·95 to 7·80. For the significance of abnormal *p*H values, and their relation to " alkali-reserve," " alkali-tolerance," and carbon dioxide tension of the alveolar air, see the following table from Hawk and Bergeim.

DISTURBANCES OF ACID-BASE EQUILIBRIUM OF BLOOD.

Area	Acid-base balance	Conditions	Associated symptoms	Compensatory mechanisms
1 Uncompensated Alkali Excess *p*H 7·5—7·8	($BHCO_3$) increased without proportionate rise in (H_2CO_3) therefore *p*H increased.	Overdosage of $NaHCO_3$. Excessive vomiting (pyloric obstruction) or gastric lavage (loss of HCl). X-ray or radium treatment.	If marked, tetany.	Diminished respiration (rise in alveolar CO_2) to hold back CO_2 Diuresis and increased $NaHCO_3$ excretion.
2—3 Uncompensated CO_2 Deficit *p*H 7·5—7·8	(H_2CO_3) decreased without proportionate fall in ($BHCO_3$) therefore *p*H increased	Hyperpnea, voluntary or induced oxygen want, (e.g. at high altitudes). Fever. Hot baths.	If marked, tetany.	Retention of acid metabolites (low NH_3 and titratable acidity of urine). Excretion of $NaHCO_3$
4 Compensated Alkali or CO_2 Excess *p*H 7·3—7·5	($BHCO_3$) or (H_2CO_3) increased but balanced by proportionate rise in (H_2CO_3) or ($BHCO_3$), therefore *p*H normal	Alkali excess. $NaHCO_3$ therapy, with slow absorption. CO_2 excess. Retarded gas exchange (e.g., emphysema) with CO_2 tension chronically increased.	Cyanosis due to deficient oxygen exchange	CO_2 retention. $BHCO_3$ retention.

Area.	Acid-base Balance	Conditions	Associated symptoms	Compensatory mechanisms
5 Normal 7·3—7·5	($BHCO_3$) and (H_2CO_3) normal at ordinary altitudes			
6 Compensated Alkali or CO_2 Deficit pH 7·3—7·5	($BHCO_3$) or (H_2CO_3) decreased but balanced by proportionate fall in (H_2CO_3) or ($BHCO_3$) therefore pH normal	Alkali deficit. Accelerated production (e.g., diabetes) or retarded elimination (e.g. nephritis) of non-volatile acids Experimental acid intoxication. Diarrheal acidosis of infancy (marasmus). CO_2 deficit. Over-ventilation at high altitudes (oxygen want).	Hyperpnea.	Increased respiration ("blowing off CO_2"). Accelerated NH_3 formation and acid excretion. Same as in Areas 2 and 3
7—8 Uncompensated CO_2 Excess pH 7·0—7·3	(H_2CO_3) increased without proportionate rise in ($BHCO_3$) therefore pH decreased	Retarded respiration as in pneumonia (physical obstruction) or morphine narcosis (deadening of respiratory centre). Experimental re-breathing. Cardiac decompensation.	Dyspnea	Increased respiration. Accelerated NH_3 formation and acid excretion. Probable shift of acid from blood to tissue.
9 Uncompensated Alkali Deficit pH 7·0—7·3	($BHCO_3$) decreased without proportionate fall in (H_2CO_3) therefore pH decreased	Terminal stages of nephritic acidosis, and diabetic acidosis (compensated by insulin therapy). Deep ether anæsthesia. Certain cardiac cases. Eclampsia.	Dyspnea	Increased respiration. Increased acid excretion and NH_3 formation (except probably in nephritis).

Principle of the method

The colorimetric method of Cullen[3], as modified by Hawkins[4], has been adapted for use with the Lovibond Comparator. Blood is collected with precautions against loss of CO_2. The plasma is diluted with saline solution containing phenol red, and the colour obtained is compared with permanent Lovibond glass colour standards.

Reagents required

1. Saline solution—Sodium chloride, 0·9% solution
2. Saline Indicator solution—2·5 ml. phenol red (0·02%) made up to 50 ml. with above saline solution, and adjusted to pH 7·4 by stirring with a glass rod dipped into 0·02N sodium hydroxide. Must be freshly made.
3. Neutral mineral oil

The Standard Lovibond Comparator Discs 5/18A and 5/18B

Disc A covers the range 7·0—7·4 pH in 9 steps of 0·05.
Disc B covers the range 7·4—7·8 pH in 9 steps of 0·05.

Technique

Place in one standard test tube 5 ml. of the saline solution, and in the other 5 ml. of the saline indicator solution. Cover each with 1 cm. layer of neutral mineral oil. Connect a Luer needle adapter by means of a short piece of rubber tubing to a 1 ml. Mohr pipette. Without stasis, insert the needle into the subject's vein, attach the adapter and allow the blood to fill the pipette past the zero mark. (A tourniquet may be used to aid in finding the vein, but the blood should flow into another tube for a few seconds after the release of the tourniquet, so that stasis is absent when the adapter is inserted into the needle). Withdraw the needle from the vein, and at once disconnect the rubber tubing from the pipette. Wipe the tip, and introduce into each tube, under the oil, 0·4 ml. of the blood. Stir carefully with a fine glass rod. Centrifuge both tubes at about 1,500 r.p.m. for 5 minutes, then withdraw the tubes and place them in the Lovibond Comparator, the tube containing the indicator being placed in the right-hand aperture, and the " blank " being placed in the left-hand aperture so that it comes behind the glass colour standard. This " blank " compensates for the slight colour and turbidity of the plasma. Hold the comparator facing a uniform source of white light—a north window when possible is best—and rotate the disc until the colour of the liquid is matched by one of the glass colour standards. Note the reading shown in the indicator recess at the bottom right-hand corner : also obtain the temperature of the saline-indicator-plasma. It is desirable to make determinations at 20°C., by placing the plasma tubes in a large beaker of water at 20°C. before observing the colour.

Calculation

The *p*H of human blood at 38°C. is obtained from the equation

$$p\mathrm{H}_{38^\circ} = \text{Colorimetric } p\mathrm{H}_{t^\circ} + 0{\cdot}01\,(t^\circ\text{—}20) - 0{\cdot}23$$

where t° = the observed temperature.

If the tubes are adjusted to 20°, the middle factor automatically drops out. The empirical correction —0·23 (" Cullen correction ") is applied to adjust the colorimetric *p*H values to those determined electrometrically at 38°C., and compensates for the protein and salt errors in the colorimetric determination.

Note

When using the Special-Purposes Lovibond Comparator for this test, 13.5 mm. cells should be employed, as the final volume of the solution is not great enough to give sufficient height in the test tubes to cover the aperture.

References

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2. J. P. Peters and D. D. Van Slyke, "*Quantitative Clinical Chemistry*", Bailliere Tindall and Cox, London, 1931
3. G. E. Cullen, *J. Biol. Chem.*, 1922, **52**, 501
4. J. A. Hawkins, *J. Biol. Chem.*, 1923, **57**, 493

The Determination of the *p*H of Nickel Plating Solutions

Introduction

The optimum working conditions for nickel-plating depend on various factors such as the composition and concentration of the plating solutions, the temperature, the degree of acidity (*p*H value), the class of work, the desired finish, and the current density employed. One of the most important of these conditions is the *p*H value, and the character of the deposit is largely dependent upon it.

Principle of the method

The *p*H of plating solutions can be determined colorimetrically by means of the appropriate indicator. The most usual *p*H range for nickel plating baths is from 5.2 to 6.8. The indicator recommended for this *p*H range is bromo-cresol purple. The deep green of the nickel solution makes it difficult to match the colour of the indicator using the normal disc for bromo-cresol purple. Two special discs have therefore been prepared for this *p*H range for use with the two bath compositions commonly employed for plating.

Reagent required

B.D.H. Bromo Cresol Purple.

The Standard Lovibond Comparator Discs 2/3A and 2/3B for *p*H range 5.2—6.8

Disc A is for use with solutions containing about 120 grams of crystalline nickel sulphate per litre.

Disc B is for use with solutions containing from 200 to 250 grams of crystalline nickel sulphate per litre.

Technique

Two standard test tubes are filled to the 10 ml. mark with the nickel solution and 0.5 ml. of the indicator solution is added to one of them. This tube, when the contents are mixed, is placed in the right-hand compartment of the comparator. The other tube is placed in the left-hand compartment and the appropriate disc for the solution being tested is placed in position in the lid of the comparator. The two solutions are then viewed through the windows in the front of the comparator and the disc rotated until the nearest colour match is obtained. The *p*H value is then read from the indicator recess in the right-hand bottom corner of the case.

Notes

1. **Other *p*H ranges.** For plating solutions with a higher degree of acidity (lower *p*H) than *p*H 5.2, bromo-cresol green indicator, with a range of *p*H 3.6 to 5.2, can be used in conjunction with an ordinary bromo-cresol green disc 2/1C.

2. **Salt error.** Electrometric methods for the determination of *p*H values of nickel plating solutions give values differing by several decimal points from those obtained by colorimetric methods. This arises from the high concentration of nickel salts in solution affecting the colorimetric method and giving rise to apparent *p*H values which are 0.2 to 0.6 *p*H units higher than the values measured on the same solutions using a hydrogen or quinhydrone electrode. For recording absolute *p*H values the electrometric methods are unquestionably the more accurate. However, for all practical purposes, the colorimetric method is more suitable for factory use as it can be performed rapidly by any ordinary workman and produce a direct reading without any need for further calculation. The results should be recorded as "apparent *p*H values by the colorimetric method."

The Assessment of the Surface *p*H of Paper

using the Lovibond-Patra *p*H Index

Introduction

The *p*H of a paper, which is the chemist's way of describing its acidity or alkalinity, is a critical factor in its response to printing. Excess acid or alkali has been shown to cause some colours to fade, and set-off and smudging can nearly always be traced to paper acidity (low paper *p*H)[1]. Printers must therefore be able to gauge the *p*H of a paper with reasonable accuracy if they are to avoid drying and fading faults.

An established method[2] of measuring the *p*H of paper involves preparing a hot-water extract of the paper, adding a chemical indicator, and comparing the colour of the solution with permanent glass standards in a Lovibond *p*H Comparator. This extraction is time consuming and will only give reliable results in the hands of skilled operators.

In an attempt to speed up the standard test, the direct application of the indicator to the paper, followed by comparison of the colour of the "smear" with the standard Lovibond disc glasses for the particular indicator used, has been suggested. This short cut has, unfortunately, been found to be unreliable, as the colour of a stain for any particular *p*H does not correspond to that of the solution of the same *p*H. This is due to the dichroic nature of the indicator solutions.

Research carried out at the Printing, Packaging and Allied Trades Research Association, (PATRA),[3] has led to the development of the present method of assessing the surface *p*H of paper. As this method will often give results which differ from those obtained by the standard hot-extract method, a special index has been devised to avoid confusion between results from the two techniques. This is called the Lovibond-PATRA *p*H Index. The *p*H values in this index range from 10 to 200, each step of 10 in the index representing a *p*H difference of about 0·2 units. Figures below 100 represent increasing acidity while those above 100 represent increasing alkalinity. The value 100 is analagous to an indicator smear colour of *p*H 4·6, which has been found to be roughly equivalent to a hot extract value of 5·0.

Probably the greatest use of the new *p*H index will be in connection with ink drying problems. It has been shown[3] that, as an approximation, uncoated papers should have a Lovibond-PATRA index of 100 or above. Similar specifications could be built up for other paper *p*H requirements. The new test should also prove satisfactory for the routine quality control of paper production, once the individual manufacturer has established his own correlation between smear *p*H index and the hot-extract standard *p*H test results.

Principle of the method

A drop of indicator is smeared, by means of a microscope slide, over the surface to be tested. The colour of the centre of the resulting smear is compared with Lovibond permanent glass standards.

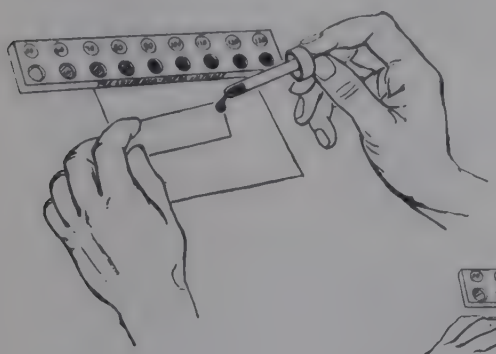

Fig. 1

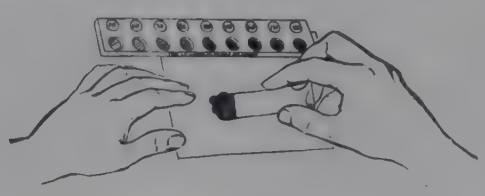

Fig. 2

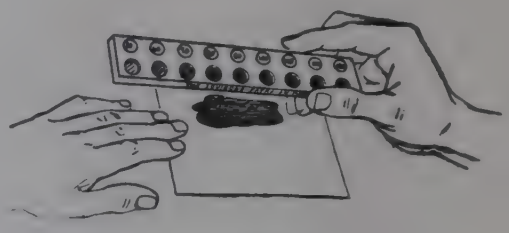

Fig. 3

Reagents required

Bromophenol Blue	Standard *p*H range	2·8–4·4)	Index	10–90
Bromocresol Green	(„	„ „	3·6–5·2)	Index 50–130
Bromocresol Purple	(„	„ „	5·2–6·8)	Index 130–200

The Standard Lovibond-PATRA *p*H Colour Slides

The specially matched colour standards of the Lovibond-PATRA Scale are mounted in black anodised aluminium slides. Three slides are available, one corresponding to each of the above indicators and covering the ranges quoted. It will be seen that there are overlaps between these ranges. The numbers however are continuous, so that the same index value obtained with different indicators represents the same *p*H value.

Technique

Place a drop of the Bromocresol Green indicator on the narrow edge of a clean microscope slide (Figure 1). Draw this across the paper to produce a smear about $3\frac{1}{2}''$ long by 1″ wide (Figure 2). If the paper is very smooth or non-absorbent, apply only light pressure so that the smear is always of approximately the same size. Allow about a minute for the indicator film to soak in, and then compare the central, uniform, area of colour in the smear with the standard glasses in the slide (Figure 3). In order to compensate for any inherent colour in the paper the standards should be viewed against a clear area of the test sheet. North daylight should be used for the comparison wherever this is possible.

If the colour of the smear is found to be at the extreme end of the Bromocresol Green slide the test should be repeated with either Bromophenol Blue or Bromocresol Purple, depending on whether the colour is below or above the standards in the slide.

Note

This test is designed as a quick test for routine spot checks on the surface *p*H of paper, and for the applications discussed above. It is not intended to replace the standard test[2].

References

1. R. R. Coupe *et al*, Printing, Packaging & Allied Trades Research Association, Interim Reports Nos. 84a, b, c & d
2. The British Standards Institution, B.S. 2924, 1957
3. D. H. Charlesworth and R. R. Coupe, PATRA Lab. Report No. 31, July 1960

The Determination of the *p*H of Soils

Introduction

One of the most perplexing and difficult problems for farmers, nurserymen, and gardeners, is to decide whether their land needs liming, and also how often liming is necessary. " Sour " soil requires liming, and " sour " soil has an acid reaction i.e. a *p*H value below 7.0. Lime ensures an alkaline reaction i.e. a *p*H value above 7.0. The determination of the *p*H of the soil, therefore, is of paramount importance, and affords an efficient and indispensable substitute for the old " hit and miss " experimental methods. Normally, the best crops grow on soil that gives an alkaline reaction, but special crops or local conditions may call for different treatment. The best turf for lawns, golf courses etc., thrives on a slightly acid soil and consequently the land is dosed with ammonium sulphate to give the required acid reaction. In either case an exact knowledge of the reaction of the soil simplifies treatment.

It is impossible to say from the *p*H value how much lime will be required. That will depend to a large extent upon the type of soil, but the horticulturist who knows his land will, after a few trials, be able to judge the needs of his particular soil from its *p*H value. In particular it will enable him to know with certainty the parts of his land which need the lime and those which already contain a sufficiency.

Principle of the method

A sample of the soil is extracted with distilled water and the *p*H value determined colorimetrically by means of a suitable indicator.

Reagent required

B.D.H. Soil Indicator

The Standard Lovibond Comparator Disc 2/1N

The disc covers the *p*H range 4.0 to 8.0 in 9 steps of 0.5.

Technique

Break up the soil into small pieces and one third fill a shaking bottle with it. Add an equal volume of distilled water, cork the bottle, and shake until the soil is evenly distributed through the liquid. Allow to settle.

An alternative solvent advocated by some workers in place of distilled water is a one-hundredth Molar solution of calcium chloride. This is used as one volume of soil sample to two and a half times its volume of the solution. This will give a result up to 0.5 *p*H units lower than a distilled water extract, but is considered to give a truer picture of the actual conditions in the soil in contact with the roots of the plants.

Decant the contents of the shaking bottle on to a filter paper in a filter funnel and collect the clear filtrate in the two standard test tubes to the 10 ml. mark.

Place one of the filled test tubes in the left-hand compartment of the comparator; to the other filled test tube add, by means of a graduated pipette, 0.5 ml. of the B.D.H. Soil Indicator. Mix the liquids and place the tube in the right-hand compartment of the comparator. Close the comparator and revolve the standard disc until the two colours viewed through the windows appear alike, or the colour of the solution falls between two of the standard colours. The *p*H of the solution is then given by the figure showing in the indicator recess in the front of the comparator.

Notes

1. For fuller details of *p*H and other chemical tests in connection with horticulture see:—
" The Chemical Testing of Plant Nutrient Solutions" by Fawcett and Stoughton
and " Chemical Tissue Tests for determining the Mineral Status of Plants in the Field" by D. J. D. Nicholas.

Both of these books are published by The Tintometer Ltd., Salisbury.

2. For convenience in carrying out the determination of soil *p*H in the field the necessary equipment can be provided in the form of a convenient kit, details of which may be obtained from The Tintometer Ltd., Salisbury.

The Determination of *p*H of Urine

using Universal Indicator

Introduction

One of the main functions of the kidneys is to maintain the acid-base equilibrium of the blood. Acids are constantly being formed as carbon-dioxide is produced by the oxidation of foodstuffs, and as phosphoric and sulphuric acids are produced by the oxidation of phosphorus and sulphur contained in ingested protein. On the other hand vegetable foods contain basic ions such as sodium, potassium and calcium which tend to make the reaction of the blood more alkaline. By the appropriate excretion of acidic or basic radicles the kidneys maintain the *p*H of the blood within narrow limits. When alkalæmia tends to occur, alkaline urine is excreted; in acidæmia, acid urine is excreted. The extreme range of *p*H changes in the urine which healthy kidneys can achieve is from 4.8 to 7.9.

Acid Urine

Freshly voided urine is usually slightly acid if the subject is eating an ordinary mixed diet. The degree of acidity is increased by a high protein intake. Marked acidity of the urine occurs in uncontrolled diabetes mellitus when aceto-acetic and hydroxybutyric acids are formed and eliminated in the urine. Very acid urines are also formed in such conditions as emphysema and chronic bronchitis, when the inadequate ventilation gives rise to a respiratory acidosis.

In the treatment of urinary infections, the urine may be made acid by giving ammonium chloride, or calcium or ammonium mandelate. The optimal degree of acidity in a freshly voided urine sample is *p*H 5.0.

Alkaline Urine

The reaction of urine may become alkaline after a meal—the so-called alkaline tide caused by the secretion of hydrochloric acid into the stomach. The reaction of the urine also tends to be alkaline on a vegetarian diet. A strongly alkaline urine is often found when there is infection of the renal tract with urea-fermenting organisms, such as *B. Proteus,* which produce ammonia. An alkaline urine is excreted in respiratory alkalosis such as occurs in hysterical hyperventilation, in which the patient exhales large amounts of carbon-dioxide. The urine is also alkaline in conditions which cause metabolic alkalosis such as vomiting in pyloric stenosis and the ingestion of large amounts of alkali.

Urine is deliberately made alkaline in the treatment of certain urinary infections and during sulphonamide therepy by giving sodium or potassium citrate or bicarbonate. The night urine is the most difficult to make alkaline and when the *p*H is greater then 7.0 sufficient alkalis are being given.

Principle of the method

The *p*H of the freshly voided urine is determined with B.D.H. Universal Indicator, the colour produced being compared with a series of Lovibond permanent glass standards representing the colours produced by solutions of known *p*H value.

Reagent required

B.D.H. Universal Indicator.

The Standard Lovibond Comparator Disc 2/1 P

This covers the *p*H range from 4.0 to 11.0 in steps of 1.0.

Technique

Fill both test-tubes or cells to the 10 ml. mark with the freshly voided urine. To the right-hand tube only add 0.2 ml. B.D.H. Universal Indicator. Do not immerse the tip of the pipette beneath the surface of the urine. Carefully mix by stirring with a clean glass rod or by pouring into another tube and back again. Hold the comparator about 18 inches in front of the eye facing a uniform source of white light—a north window whenever possible—and rotate the disc until the colours match. The *p*H of the specimen is shown in the indicator recess at the bottom of the right-hand corner.

Part 3

Chemical Analysis

Inorganic

Summary of Tests in Part 3, & Equipment Required for Each Test

Determination of	Using	Disc No.	Screen	Cells	Instrument	Equipment required
Aluminium	Haematoxylin	3/15	Yes	13.5 mm.	Comparator	Pipettes (1, 2, 50 ml.) 100 ml. beaker or flask
		NX	—	50 ml.	Nessleriser	Pipette (10 ml. graduated 0-10) measuring cylinder
Ammonia	Nessler's reagent	NAA	—	50 ml.	Nessleriser	Pipette (2 ml.)
		NAB	—	50 ml.	Nessleriser	Pipette (2 ml.)
		NAC	—	50 ml.	Nessleriser	Pipette (2 ml.)
		NAD	—	50 ml.	Nessleriser	Pipette (2 ml.)
		5/9A	—	13.5 mm.	Comparator	Pipettes (10 ml. graduated 0-10); " Permutit " test tubes
		5/9B	—	13.5 mm.	Comparator	Pipettes (10 ml. graduated 0-10); " Permutit " test tubes
Bismuth	Potassium iodide	NBA	—	50 ml.	Nessleriser	Pipettes (5 ml. graduated, 0.5, 25 ml.)
		NBB	—	50 ml.	Nessleriser	Pipettes (5 ml. graduated, 0.5, 25 ml.)
Bromide	Gold chloride	5/23	—	13.5 mm.	Comparator	Pipettes (10 ml. graduated 0-10) test tube; funnel; filter paper
Bromine	Diethyl *p*-phenylene diamine	3/53A	Yes	13.5 mm.	Comparator	
		3/53B	Yes	13.5 mm.	Comparator	
Chlorine	Neutral *o*-tolidine	3/25A	Yes	40 mm.	Special-Purposes Comparator	Pipette (1 ml.)
		3/25B	Yes	13.5 mm.	Comparator	Pipette (0.5 ml.)
	Acid *o*-tolidine	3/2A	—	13.5 mm.	Comparator	Pipette (0.1 ml.)
		3/2B	—	13.5 mm.	Comparator	Pipette (0.1 ml.)
		3/2AB	—	13.5 mm.	Comparator	Pipette (0.1 ml.)
		KDD	—	13.5 mm.	Comparator	Pipette (0.1 ml.)
		3/2APC	—	5 mm.	Comparator	Pipette (0.1 ml.)
		3/2APA	—	40 mm.	Special-Purposes Comparator	Pipette (0.2 ml.)
		3/2APB	—	40 mm.	Special-Purposes Comparator	Pipette (0.2 ml.)
		NCA	—	50 ml.	Nessleriser	Pipette (0.5 ml.)
		NCB	—	50 ml.	Nessleriser	Pipette (0.5 ml.)
		NCAB	—	50 ml.	Nessleriser	Pipette (0.5 ml.)
	Diethyl *p*-phenylene diamine	3/40A	Yes	13.5 mm.	Comparator	
		3/40B	Yes	13.5 mm.	Comparator	
		NPD	Yes	50 ml.	Nessleriser	
	Potassium iodide	3/21	—	13.5 mm.	Comparator	Pipette (0.5 ml.) litmus paper
		3/2ARP	—	40 mm.	Special-Purposes Comparator	Pipettes (1 and 50 ml.); 100 ml. beaker or flask; litmus paper

Determination of	Using	Disc No.	Screen	Cells	Instrument	Equipment required
Chlorine Dioxide	Diethyl-*p*-phenylene diamine	3/40A	Yes	13.5 mm.	Comparator	Pipette (0.1 ml.)
		3/40B	Yes	13.5 mm.	Comparator	Pipette (0.1 ml.)
Chromium	Diphenylcarbazide	—	—		Tintometer	Balance, pipettes (2, 25, 50 ml.); measuring cylinder; (100 ml.) flasks (400 ml., 250 and 500 ml. graduated) heat
Cobalt	Nitroso-R-salt	NTA	—	50 ml.	Nessleriser	Balance, pipettes (1, 2, 5 ml.); heat
		NTB	Yes	50 ml.	Nessleriser	Balance, pipettes (1, 2, 5 ml.); heat
	Thiocyanate	3/31	—	13.5 mm.	Comparator	Balance, pipette (10 ml.); separating funnel
Copper	Zinc dibenzyldithio-carbamate	3/39	—	13.5 mm.	Comparator	Pipette (10 ml.); separating funnel
	Dithio-oxamide	NDA	—	50 ml.	Nessleriser	Pipettes (0.5, 1, 5 ml.)
		NDB	—	50 ml.	Nessleriser	Pipettes (0.5, 1, 5 ml.)
		NDAB	—	50 ml.	Nessleriser	Pipettes (0.5, 1, 5 ml.)
	Sodium diethyldithio-carbamate	3/5	—	13.5 mm.	Comparator	Pipette (10 ml. graduated 0-10); separating funnel
	„ (B.N.F.M.R.A. test)	3/5	—	13.5 mm.	Comparator	Special extractor, pipettes (5, 100 ml.), funnel
	Copper sulphate	3/16	Yes	5 mm.	Comparator	
Fluoride	Zirconium-alizarin	NOA	Yes	50 ml.	Nessleriser	Pipettes (0.15, 5 ml.), 100 ml. Nessleriser glass
Iron	Thioglycollic acid	3/6	—	13.5 mm.	Comparator	Pipettes (0.5, 1, 5 ml.)
		APFE	—	40 mm.	Special-Purposes Comparator	Pipettes (0.5, 1, 5 ml.)
		NEA	—	50 ml.	Nessleriser	Pipettes (0.1, 2 ml.)
		NEB	—	50 ml.	Nessleriser	Pipettes (0.1, 2 ml.)
		NEAB	—	50 ml.	Nessleriser	Pipettes (0.1, 2 ml.)
	Thiocyanate	3/11	—	13.5 mm.	Comparator	Pipettes (5, 10 ml.); separating funnel
Lead	Dithizone	5/17	—	13.5 mm.	Comparator	Pipettes (0.1, 0.5, 1, 10 ml.); separating funnel; centrifuge; filter paper; flask (5 ml. graduated); beaker (500 ml.)
	Sodium sulphide	NF	—	50 ml.	Nessleriser	Pipettes (1, 5, 10 ml.)
Magnesium	Titan Yellow	3/28	Yes	13.5 mm.	Comparator	Pipette (5 ml. graduated 0-5)

Determination of	Using	Disc No.	Screen	Cells	Instrument	Equipment required
Manganese	Formaldoxime hydrochloride	3/21	—	13.5 mm.	Comparator	Pipettes (1, 5, 10 ml.); separating funnel; flask (50 ml.); steam bath; measuring cylinder
	Sodium bismuthate	NG	Yes	50 ml.	Nessleriser	Balance; measuring cylinder
Nickel	Ammonium citrate	3/14	Yes	13.5 mm.	Comparator	Pipette (5 ml. graduated 0-5)
	Dimethyl glyoxime	3/36	—	13.5 mm.	Comparator	Pipettes (1, 2, 5, 10 ml.); (platinum crucible & heat for solid samples)
Nitrate	Phenol-2:4-disulphonic acid	3/17	—	13.5 mm.	Comparator	Porcelain dish; water-bath; heat; pipettes (1, 5, 10 ml.); flask (25 ml. graduated)
		NHP	—	50 ml.	Nessleriser	Porcelain dish; water-bath; heat; pipettes (1, 5, 10 ml.)
	2:4-xylon-1-ol	NH	—	50 ml.	Nessleriser	Pipettes (5 ml. graduated, 10 ml.); distillation apparatus; heat
	Brucine	3/32	Yes	13.5 mm.	Comparator	Pipettes (0.1, 1, 2 ml.)
Nitrite	Griess-Ilosvay reagent	NJ	—	50 ml.	Nessleriser	Pipette (2 ml.)
Nitrogen	Copper acetate	3/2"	Yes	1 inch	Special-Purposes Comparator	Pipettes (2, 20 ml.)
Oxygen	Indigo-carmine	NOE	—	50 ml.	Nessleriser	Special glass reaction vessel; dropping pipette
	Manganese hydroxide (Winkler's method)	3/3	—	13.5 mm.	Comparator	Pipette (2 ml.); stoppered bottle (200 ml.); file
		NKA	—	50 ml.	Nessleriser	Pipettes (0.5, 2 ml.); stoppered bottle (200 ml.); file
		NKB	—	50 ml.	Nessleriser	Pipettes (0.5, 2 ml.); stoppered bottle (200 ml.); file
	Manganese hydroxide (Weir's method)	NYA	—	50 ml.	Nessleriser	Pipettes (2, 10, 20 ml.); stoppered bottle (1,110 ml.); separating funnel; file
		NYB	—	50 ml.	Nessleriesr	Pipettes (2, 10, 20 ml.); stoppered bottle (1,110 ml.); separating funnel; file
Phosphate	Ammonium molybdate & stannous chloride	3/7	—	13.5 mm.	Comparator	Pipettes (0.15, 1, 10 ml.); measuring cylinder (50 ml.); water-bath; heat; thermometer
		NMB	Yes	50 ml.	Nessleriser	Remaining apparatus as for Comparator
	Ammonium molybdate & Metol	3/4	—	13.5 mm.	Comparator	Pipettes (1, 5 ml.); water-bath; heat
		NMC	—	50 ml.	Nessleriser	Pipettes (5, 25 ml.); water-bath; heat
	Amino-naphthol-sulphonic acid	5/14	—	13.5 mm.	Comparator	Pipettes (graduated 0-1 ml.; 0-10 ml.); funnel; filter paper; test tubes

Determination of	Using	Disc No.	Screen	Cells	Instrument	Equipment required
Phosphate	Ammonium molybdate & hydroquinone	3/12	—	13.5 mm.	Comparator	Pipettes (1, 2, 5 ml.); funnel; filter paper; Nessler tubes; thermometer
	Ammonium molybdate & ammonium vanadate	3/38	—	13.5 mm.	Comparator	Pipettes (1, 5 ml.)
	Ammonium molybdate & ascorbic acid	3/51	—	13.5 mm.	Comparator	Pipettes, (4, 15, 20, 25 ml.); flask (50 ml. graduated, 100 ml. graduated); beaker (100 ml.); balance; watch-glass; heat
Silica	Ammonium molybdate	3/13	—	40 mm.	Special-Purposes Comparator	Pipettes (3, 25 ml.); flask (50 ml.); thermometer; heat
		NN	—	50 ml.	Nessleriser	Pipette (6 ml.); thermometer; heat
	1-amino-2-naphthol-4 sulphonic acid	NV	—	50 ml.	Nessleriser	Pipettes (1, 2, 4, ml.); thermometer; heat
Silver	Sodium sulphide	3/41	—	13.5 mm.	Comparator	Measuring cylinder; pipette (1 ml.)
Sodium	Manganous uranyl acetate	3/42	Yes	13.5 mm.	Comparator	Pipettes (1, 5, 10, 25 ml.); beakers (30,100 ml.); sintered crucible; filter flask; suction; ice; heat; flask; (50 ml. graduated)
Sulphite	Potassium iodide-iodate	NOB	Yes	50 ml.	Nessleriser	Pipettes (0.5, 1, 25 ml.)
Sulphur	Lead acetate	3/22	—	Paper	Special-Purposes Comparator	Filter paper; special streaming apparatus; hydrogen
Tin	Dithiol	3/35	—	13.5 mm.	Comparator	Platinum crucible; heat; measuring cylinder; pipettes (0.5, 1, 2, 10 ml.); flask (20 ml. graduated); water-bath; thermometer
Titanium	Hydrogen peroxide	3/26	—	5 mm.	Comparator	Balance; platinum crucible; heat; measuring cylinder; pipettes (10, 50 ml.); flasks (100, 500 ml. graduated)
		NRA	—	50 ml.	Nessleriser	
		NRB	—	50 ml.	Nessleriser	
		NRC	—	50 ml.	Nessleriser	
Vanadium	8-Hydroxyquinoline	3/20	—	13.5 mm.	Comparator	Pipettes (0.2, 10, 50 ml.); separating funnel; measuring cylinder

The Determination of Aluminium (1)

using haematoxylin

Introduction

The determination of residual aluminium after flocculation with aluminium salts is of great importance in water treatment, not only to make certain that the coagulation process is proceeding satisfactorily, but also to avoid wastage of chemicals. This method is applicable to waters which have been softened by the lime-soda method or to those supplies, mostly of moorland origin, treated with aluminium salts for the removal of colour and/or suspended matter. These are the main classes of waters of which it is required to determine the alumina content.

Treated waters with high hardness values may show interference, but it has been found that satisfactory results are obtained with hardness figures due to calcium salts up to 10 grains per gallon and due to magnesium salts up to 20 grains per gallon, both expressed in terms of $CaCO_3$. However it is seldom necessary in practice to know the aluminium content of such water.

Principle of the method

Haematoxylin gives a violet-purple lake with aluminium in slightly basic solution. On acidification the lake is stabilised and the colour of the excess dye changes from red to yellow.

Reagents required

1. Haematoxylin Microscopic Stain, 0.1 g. tablets, made by Messrs. Geo. T. Gurr Ltd., London.

   To 20 ml. freshly boiled distilled water in a suitable flask, add 1 tablet of the haematoxylin while the water is still hot, swirl gently to dissolve, cool, and make up to 100 ml. with distilled water. This solution should be kept in a dark bottle and not used when more than 3 days old.

2. Ammonium Carbonate (analytical reagent grade), 15 g. in 100 ml. distilled water. The loss of ammonia from this solution, or from the solid ammonium carbonate used in its preparation, must be avoided. It is suggested that the ammonium carbonate should be obtained in 100 gram bottles from fresh stock, and the whole contents dissolved in 666 ml. of distilled water. This solution should then be poured into small glass-stoppered bottles (say 50 or 100 ml.) which are kept tightly closed until required for use.

3. Acetic Acid 30% solution. 30 ml. glacial acetic acid made up to 100 ml. with distilled water.

The Standard Lovibond Comparator Disc 3/15

The disc covers the range 0.1 to 0.8 parts of aluminium per million, in steps of 0.1, while the ninth standard, labelled "blank," matches the colour of the reagents alone i.e. equals 0 parts of aluminium per million.

A dulling screen must be used with this disc.

Technique

The details must be followed exactly as departures therefrom will lead to inaccuracies.

To 50 ml. of the water under examination add 2 ml. of the ammonium carbonate solution, followed by 1 ml. of the haematoxylin solution, mix thoroughly and allow to stand for 10

minutes. Add 2 ml. of the acetic acid solution, mix thoroughly, and stand for 5 minutes. Transfer to the test tube supplied, and place in the right-hand compartment of the comparator. A "blank" of the water under test is placed in a test tube in the left-hand compartment, to compensate for any inherent colour.

Hold the comparator facing a uniform source of white light—a north window whenever possible is best—and compare the colour produced in the test solution with the colours in the standard disc, rotating the latter until a match is obtained. If the colour produced is above the highest colour value in the disc, the test should be repeated with a fresh sample, suitably diluted with distilled water. The result obtained should then be multiplied by the number of times the original sample was diluted.

The exact quantities and times above specified must be rigidly adhered to, in order to obtain accurate results. Bubbles in the solution which cling to the side of the tube render matching difficult, and should be removed by gentle tapping.

The figures on the disc represent parts of aluminium per million (= mg. per litre) when 50 ml. of the original sample are taken.

Notes

1. In order to convert the readings of the disc into parts of alumina (Al_2O_3) per million, multiply the figure obtained by the appropriate factor i.e. parts of aluminium per million $\times$ 1.89 = parts of alumina per million.

2. Interference from manganese causes bleaching, and from iron causes " off shades " (usually greenish). If more than 0.2 p.p.m. of manganese is present, the colour of the haematoxylin is bleached within 2 minutes i.e. before the addition of the acetic acid. Iron less than 0.4 p.p.m. or manganese less than 0.2 p.p.m. will allow satisfactory aluminium values to be obtained.

3. This method was developed by the Alfioc Water Treatment Service of I.C.I. Ltd., to to whom acknowledgment is made.

The Determination of Aluminium (2)

using haematoxylin

Introduction

This is a more sensitive modification of the haematoxylin test, developed by Strafford and Wyatt.

Principle of the method

In this modification excess haematoxylin is removed by means of ammonium borate, the lake is stabilised by " starch-glycerite " and the colour is measured at *p*H 7.5.

Reagents required

1. Hydrochloric acid 5N
2. Ammonium carbonate solution 2N
3. Starch-glycerite solution. Mix 1g. of analytical reagent grade starch with 20 ml. of analytical reagent grade glycerol to a smooth paste in a porcelain dish. Heat over a small flame until the mixture fumes and stir continuously until the mass becomes quiescent and clear, taking care not to overheat. Cool, mix with 80 ml. of distilled water, stand overnight and decant or filter.
4. Haematoxylin solution 0.1% This solution is prepared by crushing one tablet of haematoxylin microscopic stain 0.1 g. (Geo. T. Gurr Ltd., London) and dissolving it in 100 ml. of distilled water containing 0.1 ml. of hydrochloric acid 5N.
5. Ammonium borate solution 0.8N
6. Distilled water

The Standard B.D.H. Lovibond Nessleriser Disc NX

The disc covers the range 0.5 to 4.5γ (0.0005 to 0.0045 mg.) of aluminium, (Al), in steps of 0.5γ.

Technique

Measure a suitable volume, not exceeding 30 ml. of the water under examination, which should be approximately neutral, into a Nessleriser glass and dilute to 30 ml. with distilled water. Add 1.0 ml. of hydrochloric acid 5N, mix well, and add 6.7 ml. of ammonium carbonate 2N, swirling the liquid vigorously round the glass to assist the evolution of carbon dioxide. Add 1 ml. of starch-glycerite solution, mix, add 5.0 ml. of haematoxylin solution 0.1%, mix again and cover.

At the same time place 30 ml. of distilled water in another Nessleriser glass and treat it exactly as described above for the test solution. After allowing the blank and the test solution to stand for 15 minutes, add to each 5.0 ml. of ammonium borate 0.8 N while swirling the solutions ; dilute each to 50 ml., mixing well.

Place the blank in the left-hand compartment and the test in the right-hand compartment of the Nessleriser and allow to stand for not less than two and not more than five minutes. Stand the Nessleriser before a uniform source of white light — a north window is the best — and compare the colour produced in the test solution with the colours of the standard disc, rotating the disc until the nearest match is obtained. The value shown is in thousandths of a milligram of aluminium in the original volume taken.

Notes

1. The ammonium borate removes excess haematoxylin.
2. Iron interferes quantitatively, giving a similarly coloured lake ; copper, which can be tolerated in quantities up to 10 mg., produces a purple lake which is almost completely decomposed by ammonium borate. The amounts of iron and copper found in water are best extracted as their thiocyanates by shaking out into amyl alcohol-ether mixture. Tin also interferes, and all water used in the reagents etc. should therefore be glass distilled.
3. Hydrochloric acid is used initially to break down the aluminium which may be in a colloidal or complex form, and as the reaction of the final solution (pH 7.5) is critical the amounts of hydrochloric acid and ammonium carbonate solution should be measured accurately. The " starch-glycerite " acts as a protective colloid but slow fading occurs on standing.
4. It must be emphasised that the readings obtained by means of the Nessleriser and disc are only accurate provided that Nessleriser glasses are used which conform to the specification employed when the discs were calibrated, namely that the 50 ml. calibration mark shall fall at a height of 113 mm $\pm$ 3 mm., measured internally.

Reference

N. Strafford and P. F. Wyatt, *Analyst*, 1943, **68**, 319

The Determination of Ammonia (1)

using Nessler's Reagent

Introduction

Most waters contain small quantities of ammonium compound which, on distillation give ammonia known as " free " ammonia. On the addition of a strong alkali to the concentrated sample, followed by further distillation, additional ammonia, due to the oxidation of organic matter which is present, is driven off. This distillate is known as the "albuminoid" ammonia.

All decaying matter, whether animal or vegetable, urine, and sewage contain large quantities of ammonia. On the other hand the presence of ammonia in water may be due to micro-biological action of a harmless nature. The estimation of ammonia in water is of importance in the control of water supplies and this test has been developed with that aspect in mind. It is however adaptable for other purposes.

Principle of the method

This test is based on Nessler's reagent, a strongly alkaline solution of potassium mercuric iodide (K_2HgI_4). In the presence of ammonia a reddish-brown colour is formed.

Reagent required

The published formulæ for Nessler's reagent vary considerably and when using the B.D.H. Lovibond Nessleriser discs it is important that the reagent employed should correspond with that used for standardising the colours. The following is the formula used for this purpose.

Dissolve 35 g. of potassium iodide and 12.5 g. of mercuric chloride in 800 ml. of water and add a cold saturated solution of mercuric chloride until, after repeated shaking, a slight red precipitate remains ; then add 120 g. of sodium hydroxide, shake until dissolved, and finally add a little more of the saturated solution of mercuric chloride and sufficient water to produce 1 litre. Shake occasionally during several days, allow to stand, and use the clear supernatant liquid for the tests.

The Standard B.D.H. Lovibond Nessleriser Discs, NAA, NAB, NAC and NAD

Disc NAA covers the range 1γ to 10γ (0.001 to 0.01 mg.) of ammonia (NH_3)
Disc NAB covers the range 10γ to 26γ (0.01 to 0.026 mg.) of ammonia (NH_3)
Disc NAC covers the range 28γ to 60γ (0.028 to 0.06 mg.) of ammonia (NH_3)
Disc NAD covers the range 60γ to 100γ (0.06 to 0.1 mg.) of ammonia (NH_3)

Technique

Fill one of the Nessleriser glasses to the 50 ml. mark with the water under examination, which must be free from haze, (or with distilled water) and place it in the left-hand compartment of the Nessleriser. Fill the other Nessleriser glass to the 50 ml. mark with the sample to be tested, which must not be acid, add 2 ml. of Nessler's reagent, mix, and place it in the right-hand compartment of the instrument. Stand the Nessleriser before a uniform source of white light — a north window is the best — and compare the colour produced in the test solution with the colours in the standard disc, rotating the disc until a colour match is obtained.

Notes

1. With small quantities of ammonia, from 1γ to 5γ, the colour develops slowly and in order to obtain accurate results, fifteen minutes should be allowed to elapse before matching the colour. When more than 5γ of ammonia is present, the colour should be matched five minutes after adding the reagent.

2. The markings on the discs represent the actual amounts of ammonia (NH_3) producing the colour in the test. Thus, if on adding the reagent to 50 ml. of water a colour equivalent to 5γ is produced, the amount of ammonia present in the water will be 0.1 part per million.

3. It must be emphasized that the readings obtained by means of the B.D.H. Lovibond Nessleriser and disc are only accurate provided that Nessleriser glasses are used which conform to the specification employed when the discs were being calibrated, namely that the 50 ml. calibration mark shall fall at a height of 113 mm., plus or minus 3 mm., measured internally.

References

1. British Pharmacopæia, 1953

2. Thresh, Beale and Suckling, "*The Examination of Waters and Water Supplies*", 7th Edn., J. A. Churchill Ltd., London, 1958

The Determination of Ammonia in Urine (2)

using Nessler's Reagent

Introduction

This test was introduced for use in the indirect method of estimating Mepacrine in plasma (see Part 5). It is however applicable to the estimation of ammonia in urine for other purposes.

Principle of the method

Urine contains substances (e.g. creatinine) which interfere with direct Nesslerisation for determining the ammonia content. The ammonia is therefore removed by "Permutit-Decalso" (sodium aluminium silicate). The supernatant urine is discarded, and the "Permutit" washed. The "Permutit" is then made alkaline to liberate the ammonia, which is estimated colorimetrically by means of the Lovibond Comparator and the standard blood urea discs.

Reagents required

1. 10% w/v sodium hydroxide
2. Nessler's solution, made to the following formula :—

   Dissolve 150 g. potassium iodide in 100 ml. distilled water. Transfer this solution to a litre flask and add 200 g. mercuric iodide ($Hg\ I_2$) and allow to dissolve. When solution is complete, make up to the litre mark with distilled water, and filter. Add 1 litre distilled water to this filtrate. Dilute 15 ml. of this stock solution immediately before use with 85 ml. of distilled water.
3. **"Permutit."** A convenient fineness is that which will pass through a 60 mesh but not an 80 mesh sieve. If the "Permutit" as obtained is too coarse, it may be ground gently in a mortar. Fine particles should then be removed by repeated shaking with water, and decantation of the turbid supernatant liquid. The "Permutit" can be used repeatedly. The "Permutit" collected from a day's work should be freed from any traces of ammonia by washing with 10% w/v sodium hydroxide. The "Permutit" is then washed in 20% v/v acetic acid and finally with water. It is dried in air without heat; if it is oven-dried the activity is greatly reduced.

   The activity of each new bath of "Permutit" must be established before use, and determined against the Lovibond Blood Urea discs. The method is as follows :—

Prepare three standard ammonia-nitrogen solutions

1. 10 mg. ammonia nitrogen per 100 ml. Weigh out 94.4 mg. of $(NH_4)_2\ SO_4$ and make up to 200 ml. in a graduated flask with distilled water.
2. 5 mg. ammonia nitrogen per 100 ml. Solution 1 diluted 1 in 2 with distilled water
3. 2 mg. ammonia nitrogen per 100 ml. 20 ml. of solution 1 diluted to 100 ml. with distilled water

Take 1 ml. of each of these standard solutions through the procedure described below for urine, and draw a graph showing Lovibond Comparator readings plotted against mg. ammonia-nitrogen per 100 ml.

The Standard Lovibond Comparator Discs 5/9A and 5/9B

Disc 5/9A. covers the range 20 mg. to 100 mg. urea per 100 ml. of blood, in 9 steps.

Disc 5/9B. covers the range 110 mg. to 220 mg. urea per 100 ml. of blood, in 9 steps.

Technique

In a test tube place 100 mg. " Permutit." Add 1 ml. urine (diluted if necessary) and shake for 5 minutes. Add approximately 10 ml. distilled water, shake, and allow to settle. Pour off the supernatant fluid carefully, and add another 10 ml. of water. Again allow to settle and decant. Add 1 ml. of 10% NaOH to the " Permutit," shake, and allow to stand for 5 minutes. Add 8 ml. of water and 1 ml. of Nessler's solution. Shake, place in a comparator test tube or 13.5 mm. cell in the right-hand compartment of the comparator, a "blank" of distilled water in the left-hand compartment behind the glass standards, and compare the colours by a north light, revolving the disc until a match is obtained. Estimate the ammonia present by reference to the graph prepared for that batch of "Permutit."

With dilute urines, the estimation can be done directly on 1 ml. of the urine, but with more concentrated specimens the urine will have to be diluted. The usual dilution is 1 in 2, but specimens have been met in which dilutions of 1 in 5 or even 1 in 10 have had to be made. If a dilution is used, the ammonia-nitrogen concentration as read from the graph must, naturally, be multiplied by the dilution factor.

Note

" Permutit " is the Registered Trade Mark of The Permutit Co. Ltd., London.

" Permutit-Decalso " is specially manufactured and graded for biochemical purposes.

References

1. O. Folin and R. D. Bell, *J, Biol. Chem.*, 1917, **29**, 329
2. Army Malaria Unit, *Report to Chemical and Pharmacological Sub-committee, M.R.C. Malaria Committee*, 1944, M.L.A. 58 and 83

The Determination of Bismuth

using potassium iodide

Introduction

The estimation of bismuth is of importance in the pharmaceutical and cosmetic industries, and in metallurgy, especially in connection with the refining of copper for electrical purposes.

Principle of the method

The test is based on the yellow colours produced by the reaction between a bismuth salt and potassium iodide in acid solution to form potassium bismuth iodide ($KB_2 I_4$). Under these conditions, many substances form precipitates or liberate free iodine, producing yellow solutions. Such interfering substances must consequently be removed from the solution containing the bismuth before the quantitative test can be applied. In general, chlorides, nitrates, aluminium, magnesium, zinc and small quantities of copper, lead, ferrous iron, manganese, arsenic and cadmium do not interfere. Silver, tin, antimony, mercury, nitrites and oxidising agents must be absent. In order to ensure that the yellow colour produced in the test is not wholly or in part due to the presence of an oxidizing agent liberating iodine from the acid solution of potassium iodide, a small quantity of dilute sulphurous acid is used in each test.

Reagents required

1. Sulphuric acid, approximately 5N
2. A 10% w/v aqueous solution of potassium iodide (analytical reagent grade)
3. Dilute sulphurous acid containing 0.5% SO_2 prepared by diluting sulphur dioxide solution (5% w/v SO_2) with distilled water

The Standard B.D.H. Lovibond Nessleriser Discs NBA and NBB

Disc NBA covers the range 10γ to 50γ (0.01 to 0.05 mg.) of bismuth (Bi).

Disc NBB covers the range 30γ to 200γ (0.03 to 0.2 mg.) of bismuth (Bi).

Technique

To 25 ml. of the solution under examination (or other suitable quantity) free from interfering metals, contained in a Nessleriser glass, add 3 ml. of 5N sulphuric acid and sufficient water to produce 50 ml. Then add 2 ml. of potassium iodide solution and 0.2 ml. of dilute sulphurous acid, mix and place in the right-hand compartment of the Nessleriser. In the left-hand compartment place a Nessleriser glass containing 3 ml. of 5N sulphuric acid diluted to 50 ml. with distilled water, 2 ml. of potassium iodide solution, and 0.2 ml. of dilute sulphurous acid. Stand the Nessleriser before a uniform source of white light — a north window is the best —and compare the colour produced in the test solution with the colours in one of the standard discs, rotating the disc until a colour match is obtained.

The discs are calibrated in terms of bismuth metal. If a colour equivalent to 50γ (0.05 mg.) is produced in the test, then the quantity of the solution taken for the test contains 50γ (0.05 mg.) of Bi.

If the colour obtained is deeper than the standard colours, the test should be repeated on a smaller quantity of the solution.

Notes

1. The application of the B.D.H. Lovibond Nessleriser to the determination of bismuth was suggested by B. Drinkwater, A.R.S.M., of the British Non-Ferrous Metals Research Association. The colour standards were prepared in the laboratories of the Association.

2. For the separation of bismuth from copper before carrying out colorimetric determinations of bismuth, reference should be made to the report of the Chemical Sub-Committee, Fiscal Policy Joint Committee, Brass, Copper and Nickel Silver Industries (*Analyst*, 1935, **60**, 554).

3. It must be emphasized that the readings obtained by means of the B.D.H. Lovibond Nessleriser and disc are only accurate provided that Nessleriser glasses are used which conform to the specification employed when the discs were being calibrated, namely that the 50 ml. calibration mark shall fall at a height of 113 mm., plus or minus 3 mm. measured internally.

The Determination of Bromide

using gold chloride

Introduction

Estimation of the amount of bromide in blood is of value in confirming the clinical diagnosis of bromism as manifest by a skin eruption or by mental changes. Formerly patients with mental disease were often heavily sedated with bromides and on rare occasions bromide intoxication, characterised by disorientation and hallucinations, developed. In such cases it is important to distinguish between the symptoms of bromide intoxication and those due to the underlying mental disease itself.

Blood normally contains 0.25 to 2 mg. sodium bromide per 100 ml. In patients with mental symptoms due to bromide intoxication levels of 200 mg. or more per 100 ml. are found. For the estimation 5 ml. venous blood, collected in a clean tube containing a pinch of potassium oxalate powder, are required.

Principle of the method

The proteins of serum, or plasma, are precipitated by trichloracetic acid. The filtrate is treated with gold chloride solution, which combines with bromides to give a double salt, the colour of which is a deeper yellow-brown than that of the added gold solution : the colour is compared with that of a series of glass standards prepared to match bromide standard solutions similarly treated.

Reagents required

(a) 20% w/v trichloracetic acid

(b) 0.5% gold chloride Dissolve 0.5 g. of gold chloride ($AuCl_3HCl.3H_2O$), in distilled water, and make up to 100 ml.

The Standard Lovibond Comparator Disc 5/23

The disc covers the range 0, 10, 25, 50, 75, 100, 125, 150, 175 mg. of sodium bromide per 100 ml. of serum or plasma.

Technique

Mix in a test tube:—

Serum or plasma	..	2 ml.
Distilled water	..	6 ml.
20% trichloracetic acid	..	2 ml.

Filter. To 5 ml. filtrate (=1 ml. serum) add 1 ml. of 0.5% gold chloride solution. Mix, transfer to a comparator test tube (or 13.5 mm. cell if a Special-Purposes Lovibond Comparator is being used) and place in the right-hand compartment of the comparator. Hold facing a uniform source of white light—a north window wherever possible is best—and rotate the disc until the colour of the liquid is matched by one of the glass standards. The value (expressed in mg. sodium bromide per 100 ml. serum or plasma) is then read from the indicator recess at the bottom right-hand corner of the comparator.

Notes

1. It is important to check each batch of reagents against the 0 of the disc. Prepare a blank from :—

4 ml. distilled water
1 ml. trichloracetic acid solution
1 ml. gold chloride solution

and mix well.

If this does not match the 0 of the disc, it is useless to proceed ; the gold chloride may be different from that used in standardising the disc.

2. Iodides, as well as bromides, give double salts with gold chloride. It is therefore essential to make sure that the patient is not taking iodides.

Confirmatory test

If the patient is suffering from skin lesions thought to be due to bromism, and the bromide found by the above test is low (25 mg. or less), the fluorescein confirmatory test should be performed, to ascertain that increased bromide is in fact present.

Bromides are oxidised to bromine by chloramine T. The bromine is taken up by fluorescein to yield red tetrabromofluorescein (eosin).

Reagents required for confirmatory test

(a) 0.4% Chloramine-T

(b) 0.125% fluorescein Dissolve 125 mg. of fluorescein in 25 ml. of N/10 NaOH and add distilled water to 100 ml.

(c) Buffer solution pH 5.3 to 5.4 Dissolve 6.6 grms. of anhydrous sodium acetate (or 10.9 grms. of $CH_3COONa.3H_2O$), and 1.2 ml. of glacial acetate acid in water to 100 ml.

Technique

Mix in a small test tube :—

Serum filtrate, as prepared for gold chloride test	1 ml.
Buffer solution	0.5 ml.
Fluorescein solution	1 drop
Chloramine-T	1 drop

Treat 1 ml. of distilled water, or 1 ml. of protein-free filtrate from normal serum, in the same way in parallel.

Observe the colour of the contents of the two tubes : the filtrate from the patient's serum will turn orange-red or red, in a few seconds, if more than 5 mg. NaBr is present. Observe the tubes for five minutes before declaring the reaction negative. If bromides were not in excess of normal the treated filtrate of the patient's serum will not alter in colour, but will remain like the treated water or treated normal filtrate.

Remarks

Iodide, in this test, will give tetraiodofluorescein, which is similar in colour. The iodide can be removed by treating the protein-free filtrate with sodium nitrite solution and extracting with chloroform, but it is simpler to ask whether the patient is taking iodide.

Reference

G. A. Harrison, *Chemical Methods in Clinical Medicine,* 4th Edition, J. & A. Churchill, London, 1957, page 362

The Determination of Bromine

using diethyl-*p*-phenylene diamine (Palin DPD)

Introduction

The purification, by chlorination, of water containing both bromide and ammonia leads to the formation of both monochloramine and monobromamine. These conditions may arise in the chlorination of sea water swimming pools or of water contaminated by sea water. Monobromamine may also be formed as a result of water purification using either bromine itself[1] or a brominating agent such as 1 : 3-dibromo-5 : 5-dimethyl hydantoin (DBDMH)[2]. In all these cases the estimation of free residual bromine is a necessary part of the control of the disinfection process. This method[3] has been developed for this purpose.

Principle of the method

Both bromine and monobromamine react with diethyl-*p*-phenylene diamine (DPD) to give a red colour. Chlorine is prevented from reacting by the addition of an ammonium salt which, at the appropriate *p*H, converts the chlorine to chloramine which does not give an immediate colour with DPD. The intensity of the colour which develops on the addition of DPD to the sample, in the presence of an ammonium salt and a buffer, is thus proportional to the concentration of free and combined bromine in the sample. The intensity of the colour is measured by comparison with Lovibond permanent glass standards.

The method does not differentiate between free and combined bromine, but as there does not appear to be the same difference between the chemical and bacteriological action of free and combined bromine as there is between chlorine and the chloramines, their separate determination has little, if any, practical importance.

By a simple supplementary procedure it is possible to adapt the method for the determination of bromine (free plus combined), chlorine and chloramines in the same sample.

Reagents required

DPD No. 1 tablets
DPD "NH_3" tablets
DPD No. 3 tablets

The Standard Lovibond Comparator Discs 3/53A and 3/53B

3/53A covers the range 0.2 to 2.0 parts per million bromine
3/53B covers the range 1.0 to 10.0 „ „ „ „

These discs are calibrated for use with 13.5 mm. cells or test tubes; and a dulling screen, which is common to both discs, must be used.

The master discs, against which all reproductions are checked, were tested and approved by Dr. Palin.

Technique

For total free bromine in the absence of free chlorine (Note 1)

Rinse out a 13.5 mm. cell, or test tube, with the sample to be tested, and leave in the cell just enough of the liquid to cover a tablet. Drop one DPD No. 1 tablet into this prepared tube and allow to disintegrate ; *i.e.* leave until the effervescence ceases. Add the water sample up to the 10 ml. mark, mix rapidly to dissolve the remains of the tablet, and place the cell in the right-hand compartment of the comparator. Match immediately against the standards in the disc, using north daylight wherever possible. To compensate for any inherent colour in the sample an identical cell, containing the sample only, should be placed in the left-hand compartment of the comparator. The figure shown in the indicator window of the comparator represents parts per million of free residual bromine present in the sample.

For free bromine and free and combined chlorine

The reading obtained in the preceding procedure now corresponds to free bromine plus free chlorine. This is Reading No. 1. After taking this reading add to the right-hand

cell one DPD No. 3 tablet. Mix the contents of the cell, by shaking until the tablet has dissolved, allow the cell to stand for 2 minutes and then match against the standards as before. This is Reading No. 2. The difference between Reading No. 2 and Reading No. 1 represents the concentration of combined chlorine (Note 2).

Take an indentical cell, rinse out with the water sample as before, add one DPD "NH_3" tablet and make up to 10 ml. with the water sample. Prepare a further cell containing one DPD No. 1 tablet, as in the first procedure, add the 10 ml. of sample treated with the DPD "NH_3" tablet, mix rapidly to dissolve the remains of the tablet and match immediately. This is Reading No. 3 and gives the concentration of free bromine. To obtain the concentration of free chlorine deduct Reading No. 3 from Reading No. 1 (Note 2).

Notes

1. If free chlorine is known, or suspected, to be present but only the concentration of free bromine is required, it is necessary only to obtain Reading No. 3 using DPD No. 1 and DPD "NH_3" tablets.

2. The comparator discs used in these tests are calibrated in terms of bromine. To obtain the concentrations of free and combined chlorine in the differential procedure the differences obtained must be multiplied by 0.44 thus:—

Free chlorine =0.44 (Reading No. 1 — Reading No. 3).
combined chlorine =0.44 (Reading No. 2 — Reading No. 1).

3. Dissolved oxygen in water can produce a faint colour with the reagent if the solution is allowed to stand. Provided the instructions are followed exactly there is no interference within the period of the test.

4. The only potential interference likely to be present in water is that due to oxidised manganese. This interference can be compensated by developing the manganese colour, in the blank, as follows :—

To 10 ml. of sample in a separate test tube add 1 drop of 0.5% sodium arsenite ($NaAsO_2$) solution, and mix. Rinse a comparator cell with sample and add one DPD No. 1 tablet. After effervescence has ceased add the 10 ml. of arsenite treated sample. Mix, to dissolve the remains of the tablet, and then place this cell in the left-hand compartment of the comparator. In this way the colour due to manganese will have been developed equally in both test and blank and will cancel out.

5. All glassware used must be thoroughly rinsed after making a test. Handling the tablets and unnecessary exposure to air should be avoided. The tablets should be transferred to the comparator cell by shaking into the bottle cap rather than by the use of the fingers.

6. If the colour developed is equal to, or greater than the 5 p.p.m. standard, the test should be repeated after diluting the sample with distilled water, the final reading being multiplied by the dilution factor.

References

1. *Chemical Week*, 1959, (Dec. 12), p. 90
2. R. A. Reed, *Chemical Products*, July 1960
3. A. T. Palin, *Water and Sewage Works*, in press
4. N. D. R. Schaafsma, *Water Pollution Abstracts (D.S.I.R.)*, 1949, **22**, 196
5. J. K. Johannesson, *Analyst*, 1958, **83**, 155
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The Determination of Free Available Chlorine and Chloramine (1)

using neutral *ortho*-tolidine (Palin)

Introduction

Where it is necessary to determine both free chlorine and combined chlorine in treated waters the neutral *ortho*-tolidine method is preferred to the acid ortho-tolidine method described elsewhere.

Principle of the method

The procedure consists in the treatment of the chlorine-containing solution with neutral *o*-tolidine, in the presence of stabilising agents, for the production of a blue coloration. Reaction with chloramine is activated by the addition of potassium iodide. The only interfering substance is manganese, and a method is given for making due allowance for this. Unlike the acid *ortho*-tolidine method, nitrites in this method are without effect.

Reagents required

1. Buffer stabilising solution

Dissolve 4 grams sodium hexametaphosphate (" Calgon ") in about 80 ml. distilled water, add 0.4 ml. " Teepol 530 " (Shell Chemicals Ltd.), mix, and make up to 100 ml. with distilled water.

Note—If subjected to low temperatures, this solution may become turbid, but may be clarified by warming. Cool afterwards to room temperature for use. Alternatively, the inclusion of 10% v/v acetone in the reagent will inhibit precipitation and not influence the reaction.

2. Neutral *o*-tolidine solution 0.1%

Place 1 gram *o*-tolidine (specially purified Reagent quality B.D.H.) in a mortar with 5 ml. of 20% v/v HCl. Grind to a thin paste. Dissolve in about 500 ml. distilled water, and then dilute with more distilled water to 1 litre. Store in an amber bottle. The water must be chlorine-free.

3. Potassium iodide crystals (analytical reagent grade)

The Standard Lovibond Comparator Discs 3/25A and 3/25B

3/25A covers the range 0.1 to 1.0 parts per million chlorine. (This disc must be used in conjunction with the Special-Purposes Lovibond Comparator and 40 mm. cells).

3/25B covers the range 0.25 to 5.0 parts per million chlorine. (This disc may be used in conjunction with either of the Lovibond Comparators, and requires 13.5 mm. cells or test tubes).

Both discs must be used in conjunction with a dulling screen, which is common to both discs.

The chlorine values obtained by the use of these discs are identical with those obtained by the F.A.S. titration method of Palin. The master discs against which all reproductions are checked were tested and approved by Dr. Palin.

Technique

Disc 3/25A Measure into the 40 mm. cell 1.0 ml. of reagent **1** followed by 1.0 ml. of reagent **2** ***(in that order)***. Add the water sample up to 20 ml., mix and place at once in the right-hand compartment of the Special-Purposes Lovibond Comparator, so that it comes behind the centre of the disc. In the left-hand compartment place a similar cell containing the water sample only. Hold the comparator facing a good source of diffused north daylight, and revolve the disc until the nearest match is obtained. The figure then shown in the indicator window represents the parts per million of free chlorine present in the sample. The matching must be made immediately after mixing the solutions.

Disc 3/25B Measure into the 13.5 mm. cell or test tube 0.5 ml. of reagent **1** followed by 0.5 ml. of reagent **2** ***(in that order)***. Add the water sample up to the 10 ml. mark, mix, and place at once in the right-hand compartment of the comparator, so that it comes behind the centre of the disc. In the left-hand compartment place a similar cell or tube filled with the water sample. Match at once as above. The figure shown represents parts per million free chlorine as before.

Determination of Chloramines by these Discs

Monochloramine may be determined as follows:—continue the above test by adding a small crystal of potassium iodide (analytical reagent grade) to the right-hand cell and mix well. Evaluate the colour as previously, and deduct from this the reading first obtained as above. The result represents monochloramine in parts per million Cl_2.

Dichloramine is not usually present in significant quantities if the *p*H of the water is above 7. If it is required to estimate it, proceed as follows :—

To the solution which has already been treated with reagents 1 and 2 and potassium iodide (see above) add 0.5 ml.* of 0.5% v/v sulphuric acid (analytical reagent grade) and mix. Then add 0.5 ml.* of 5% w/v sodium bicarbonate solution and again mix.

Evaluate this colour as previously, multiply this result by 1.1 to allow for the volume change, and deduct from this answer the result previously obtained with potassium iodide. The result represents parts per million Cl_2 as dichloramine.

Trichloramine. If nitrogen trichloride is present in the sample, which would be indicated by its distinct odour, it should be removed by extraction with carbon tetrachloride (analytical reagent grade).

Notes

1. The only interfering substance likely to be present in water is oxidised manganese. Its effect can be allowed for by developing the manganese colour in the " blank " as follows :—

Instead of using a " blank " cell in the left-hand compartment, containing the sample only, place 0.5 ml.* of reagent **1** in a clean cell, add a crystal of potassium iodide and 1 drop* of a 0.5% solution of sodium arsenite $(NaAsO)_2$. Make up to the 10 ml.* mark with the sample, and mix. Add 0.5 ml.* of reagent **2** and mix. Place this cell in the left-hand compartment as a blank : thus the colour due to manganese will have been developed equally in both fields, and cancels out.

2. All glassware used must be very thoroughly rinsed after making a test; this is particularly important, as only a trace of potassium iodide will cause a chloramine colour to develop.

The colour produced is reasonably stable at temperatures up to 80°F. Above this temperature, fading will occur and increase proportionately rapidly, but if the reading is taken immediately after mixing the reagents and sample, no significant error will be introduced.

*these quantities refer to a 13.5 mm. cell. For the 40 mm. cell, double these figures.

References

1. A. T. Palin, *J. Inst. Water Eng.*, 1949, **3**, 100
2. R. W. Aitken and D. Mercer, *ibid.*, 1951, **5**, 321
3. Patent No. 680427

The Determination of Residual Chlorine (2)

using acid *o*-tolidine

Introduction

The administration of chlorine to reservoirs, storage tanks, and swimming pools, and the emergency chlorination of water-mains, is an accepted practice. In correct proportions, it ensures sterility of the water by destroying *B. Coli*, removes contamination (e.g. by sewage) and prevents the growth of algae. It is necessary, however, to keep a close check on the amount of residual chlorine left in the water after its " chlorine demand " has been fulfilled, as an excess of chlorine beyond a certain point would be harmful, give a very unpleasant flavour and smell to potable waters and, in the case of swimming baths, cause discomfort to bathers through smarting eyes. The Ministry of Housing and Local Government [1, 2] recommend a "free" chlorine content of between 0.2 and 0.5 p.p.m. with marginal chlorination, and more than double this with "break point" chlorination.

Principle of the method

For low concentrations of chlorine in potable and swimming waters, use is made of the reaction with *ortho*-Tolidine, which gives a yellow colour in the presence of chlorine concentrations of the order of 5 parts per million and under. The same colour is given by nitrites in water, but if their presence is suspected a simple test is available. The yellow colour due to chlorine is destroyed by sodium thiosulphate (photographers' " hypo "), while that due to nitrites is not so destroyed. If, therefore, on the addition of a small crystal of " hypo " the yellow colour vanishes, this is confirmation that the colour was in fact due to chlorine. The test tube or cell must be carefully washed after each test, or subsequent tests would be spoilt by any residual " hypo."

Reagent required

ortho-Tolidine, (analytical reagent grade)	1 gm.
Hydrochloric acid (analytical reagent grade)	100 ml.
Redistilled water (analytical reagent grade) zero chlorine demand, to produce ..	1000 ml.

The Standard Lovibond Discs

Comparator

3/2 A	0.1 to 1.0 parts per million chlorine (Cl)	
3/2 B	1.2 to 2.0 „ „ „ „	(5 standards only)
3/2 AB	0.15 to 2.0 „ „ „ „	
KDD	0.1 0.3 0.6 0.9 1.1 p.p.m.	(5 standards only)
3/2 APC	1.0 to 5.0 parts per million chlorine	For use only with 5 mm. cells
3/2 APA	0.02 to 0.3 „ „ „ „	For use only with Special-Purposes Lovibond Comparator and 40 mm. cells
3/2 APB	0.2 to 0.8 „ „ „ „	

Nessleriser

NCA	0.01-0.09 part per million chlorine (Cl)
NCB	0.1 -0.5 „ „ „ „ „
NCAB	0.02-0.5 „ „ „ „ „

Technique

Fill both test vessels with the water to be tested and add the reagent to that in the right-hand compartment, so that the left-hand vessel contains a " blank " of the water behind the colour standard, to act as compensation for any inherent colour. Hold the comparator facing a uniform source of white light—a north window when possible is best—and rotate the disc until the colour of the liquid is matched by one of the glass standards. The value, expressed in parts per million, is then read from the indicator window.

The reagent is used in the proportion of 1 to 100. Mix the water and reagent and match immediately to obtain the figure for free chlorine. If the colour continues to develop (as indicated by a second reading at say 5 minutes) this indicates the presence of chloramines. In this case, take a reading after allowing to stand for 15-20 minutes, which indicates total residual chlorine. The difference between this and the original free chlorine reading indicates combined chlorine. Strongly alkaline water should be rendered acid, by the use of double the proper proportion of reagent, for full development of the colour.

***Discs* 3/2 APA and 3/2 APB,** must be used in conjunction with 40 mm. cells. 20 ml. of water are most conveniently employed, so 0.2 ml. of reagent is required.

***Discs* 3/2A, 3/2B, 3/2AB and KDD** Fill the test tubes to the 10 ml. mark and add 0.1 ml. of reagent.

***Disc* 3/2 APC** must be used in conjunction with 5 mm. cells. The cells hold 5 ml., so that it is more convenient to mix 10 ml. of the water with 0.1 ml. of reagent in another vessel, e.g. a test tube, and fill the right-hand cell with the mixture.

***Discs* NCA, NCB, and NCAB** Use 0.5 ml. reagent in the 50 ml. sample in a Nessleriser cylinder.

For Field Work, where portability and ease of manipulation is important, *ortho*-tolidine tablets are available in place of the standard liquid *ortho*-tolidine reagent.

Place one tablet in the test tube, and fill with the sample to the mark. Mix well and match in the usual way.

Notes

1. It must be emphasized that the readings obtained by means of the B.D.H. Lovibond Nessleriser and disc are only accurate provided that Nessleriser glasses are used which conform to the specification employed when the discs were being calibrated, namely that the 50 ml. calibration mark shall fall at a height of 113 mm. plus or minus 3 mm. measured internally.

2. For details of a Special Comparator for the limit testing of both chlorine and *p*H in water, apply to The Tintometer Ltd., Salisbury.

3. For high chlorine concentrations, up to 250 p.p.m., the potassium iodide test should be used.

References

1. *The Purification of the Water of Swimming Baths*, Min. of Housing & Local Govt. 1951, reprinted 1957

2. *The Bacteriological Examination of Water Supplies*, Report **71**, 1957, by the same authority

The Determination of Free and Combined Residual Chlorine (3)

using diethyl-*p*-phenylene diamine (Palin-DPD)

Introduction

This method is particularly useful in those cases where it is necessary to estimate nitrogen trichloride in addition to free chlorine and the chloramines. The procedure has been greatly simplified by the use of tablets. The method is suitable for use in sea-water baths as iodine and bromine do not interfere.

Principle of the method

Researches in chlorine chemistry have resulted in the development of a very simple procedure for the differential determination of residual chlorine compounds in water. Depending upon the information required, it may be adapted to give total residual chlorine, or free and combined residual chlorine or complete separation into the free chlorine, monochloramine and dichloramine fractions. For the estimation of nitrogen trichloride, rarely required in practice, a simple supplementary procedure is provided. Differentiation, which with the new DPD indicator is remarkably clear-cut, into these various forms of residual chlorine is of the greatest importance in the control of modern chlorine processes of water treatment. With free chlorine the indicator gives a red colour. Subsequent addition of a small amount of potassium iodide immediately causes monochloramine to produce a colour. Further addition of potassium iodide to provide a considerable excess evokes a rapid response from dichloramine. The colours produced require no stabilising agents and interference is suppressed by using EDTA as a chelating agent. With this method, correction of free chlorine readings for the presence of any nitrogen trichloride is unnecessary. When present, this compound is included with dichloramine, which in many of its properties it closely resembles.

A novel feature of this Lovibond Comparator method lies in the use of compressed tablets which, besides being far more convenient in use, permit of reagents being combined together to give a procedure of exceptional simplicity.

Reagents required

Comparator

DPD tablets
(a) No. 4 (or Nos. 1 & 3) for total residual chlorine
(b) Nos. 1 & 3 for free and combined residual chlorine
(c) Nos. 1, 2 & 3 for free chlorine, monochloramine and dichloramine (also nitrogen trichloride)

Nessleriser

DPD tablets
(a) Nos. 1 & 3 for total residual chlorine
(b) Nos. 1 & 3 for free and combined residual chlorine
(c) Nos. 1, 2 & 3 for free chlorine, monochloramine and dichloramine (also nitrogen trichloride)

The Standard Lovibond Discs

Comparator

3/40A covers the range 0.1 to 1.0 parts per million chlorine.
3/40B covers the range 0.2 to 4.0 parts „ „

These discs require 13.5 mm. cells or test tubes. A dulling screen, which is common to both discs, must be used.

Nessleriser

NPD covers the range 0.05 to 0.5 p.p.m. This disc must be used with a dulling screen and 50 ml. tubes.

The chlorine values obtained by the use of these discs are identical with those obtained by the FAS titration method of Palin (*Standard Methods for the Examination of Water, Sewage and Industrial Wastes*, 10th Edn., American Pub. Health Assn. 1955). The master discs against which all reproductions are checked were tested and approved by Dr. Palin.

Technique

(a) Comparator

For Total Residual Chlorine

Place in the left-hand compartment, behind the colour standards of the disc, a 13.5 mm. cell or test tube containing the sample only. Rinse a similar cell with the sample, and leave in it enough of the liquid just to cover the tablet when added. Drop into this prepared tube one No. 1 and one No. 3 tablet (or one No. 4 tablet, which is these two combined) and allow to disintegrate ; that is, until effervescence ceases. Then add the water sample up to the 10 ml. mark, mix rapidly to dissolve the remains of the tablet, and place the cell in the right-hand compartment of the comparator. Match after two minutes by holding the comparator facing good source of diffused north daylight and revolve the disc until the correct standard is found. The figure then shown in the indicator window represents parts per million of total residual chlorine present in the sample.

For differential estimation of free and combined Residual Chlorine

Prepare the tubes as above, one " blank " tube and one with just a few drops of the sample*, and to this tube add one No. 1 tablet only. After disintegration, make up to 10 ml. and mix as before and match at once. This gives free residual chlorine. Then add one tablet No. 3, mix and stand for two minutes. The colour then read off represents total residual chlorine. By deducting the first reading (free residual) the combined residual chlorine value is obtained.

For complete differentiation

Prepare the two tubes as above, and disintegrate one No. 1 tablet, make up to 10 ml. and mix. Match at once and read off free residual chlorine as above (Reading No. 1).

Next add to the right-hand cell one tablet No. 2, mix vigorously to dissolve and match at once (Reading No. 2). From this deduct Reading No. 1 to obtain monochloramine value in parts per million.

Finally add one tablet No. 3, mix vigorously, stand for two minutes and match (Reading No. 3). From this deduct Reading No. 2 to obtain dichloramine value.

Nitrogen Trichloride

Nitrogen trichloride, whose presence in the water would be indicated by its distinct odour, normally gives a colour in the dichloramine fraction of the differential test. It may be caused to produce colour in the free chlorine fraction if the sample is first treated with potassium iodide. Using this as a basis, the following method for its estimation has been evolved.

Rinse the right-hand cell in the manner prescribed above. Add one DPD tablet No. 1 and allow to effervesce for disintegration. To 10 ml. of the water sample in a separate tube add one DPD tablet No. 2 and mix vigorously to dissolve. Then add the 10 ml. of sample so treated to the right-hand cell containing the remains of tablet No. 1 and mix rapidly to dissolve. With the left-hand cell containing the water sample only, evaluate the colour as before. From the reading thus obtained deduct the free chlorine reading (and the monochloramine if any) and multiply by two. The result corresponds to *nitrogen trichloride* in parts per million chlorine. The next step is to multiply this result by 0.4 and to subtract the answer from the dichloramine reading as given by the normal differential procedure. The purpose of this is to correct the dichloramine reading for that portion of the nitrogen trichloride which appears with it. The total residual chlorine is then the sum of the various constituents. It is emphasized, however, that in practice it will usually be quite adequate to include any nitrogen trichloride with dichloramine and not to perform the further differentiation.

(b) Nessleriser

The instructions given for the comparator should be followed exactly except that 50 ml. should be substituted for 10 ml. and that special Nessleriser D.P.D. tablets should be used. There is no No. 4 Nessleriser tablet and No. 1 + No. 3 should be used instead as the very slight

*If monochloramine content is high, differentiation is improved by using distilled water instead of the sample for disintegrating the tablet.

trace of free iodine which sometimes arises during ageing of No. 4 tablets would cause an obvious colour in the greater depth of the Nessleriser cell although it is undetectable in a comparator cell.

Notes

1. When using excess potassium iodide any copper present in the water sample would be able to produce a colour with the DPD indicator. This interference (up to approx. 10 p.p.m. Cu) is prevented by the EDTA incorporated in the tablets. In the simple test for total residual chlorine, where potassium iodide is used from the start, very high concentrations of copper may give a colour until sufficient EDTA has dissolved for complete chelation. Such colours, however, are of a transitory nature and disappear within the two minute period of the test. With the differential tests any copper present is, of course, chelated before potassium iodide is added and cannot then produce colour.

2. Dissolved oxygen in water can produce a faint colour with the reagent if allowed to stand. The suppression of trace metal catalysis by the EDTA minimises this effect and there is no interference within the period of the test.

3. The only interfering substance likely to be present in water is *oxidised manganese*. Its effect can be allowed for by developing the manganese colour in the " blank " as follows :—

To 10 ml. of sample in a separate test tube add one DPD tablet No. 2 and 1 drop of 0.5% sodium arsenite ($NaAsO_2$) solution. Mix to dissolve. Rinse the comparator cell or test tube as before and add 1 DPD tablet No. 1. Allow to effervesce for disintegration and then add the 10 ml. of arsenite-treated sample. Mix to dissolve remains of tablet and then place in left-hand side of Comparator. In this way the colour due to manganese will have been developed equally in both fields and thus cancels out.

4. All glassware used must be very thoroughly rinsed after making a test, since only a trace of potassium iodide will cause chloramine colour to develop. For the same reason handling the tablets, particularly DPD No. 1, should be avoided. By shaking one tablet into the bottle top it is a simple matter to use the top for conveying the tablet to the Comparator cell.

5. The quantity of indicator used in the tablets has been chosen to suit the range of chlorine concentrations covered by the discs, that is up to 4 parts per million. Samples containing higher concentrations must be diluted. Concentrations of chlorine above 8 p.p.m. will entirely bleach the colour, and give an apparently zero reading, but at this concentration the smell of chlorine would be very apparent. If there is any doubt about the need for dilution a simple check is to repeat the procedure using two DPD No. 1 tablets instead of one. A very decided increase in colour would indicate dilution to be necessary, in which case the requisite amount of distilled water is added first to the reagent followed by the measured amount of sample.

6. For details of a special portable set for use with this test, apply to The Tintometer Ltd., Salisbury.

7. It must be emphasised that the readings obtained by means of the B.D.H. Lovibond Nessleriser and disc are only accurate provided that Nessleriser glasses are used which conform to the specification employed when the discs were calibrated, namely that the 50 ml. calibration mark shall fall at a height of 113±3 mm. ; measured internally.

References

1. A. T. Palin, *Analyst*, 1945, **70**, 203
2. A. T. Palin, *Jour. Amer. Water Wks. Assn.*, 1957, **49**, 873
3. A. T. Palin, Paper presented to the Society for Water Treatment and Examination, October, 1957
4. A. T. Palin, *Baths Service*, 1958, **17**, 21
5. A. T. Palin, *Water and Water Eng.*, 1958, **62**, 30
6. A. T. Palin, *Archiv. des Badewesens*. 1958, August, 216

The Determination of Residual Chlorine (4)

using potassium iodide

Introduction

Chlorine is used in high concentrations for certain industrial purposes (e.g. bleaching and washing processes) and for emergency water-main treatment. For high chlorine concentration the *ortho*-tolidine colours are too intense for accurate discrimination, and the following method has been adopted for use in such cases.

Principle of the method

The method is based on the reaction of chlorine with potassium iodide in acid solution to liberate iodine. The colour of the iodine is matched against Lovibond permanent glass standards.

Reagents required

1. Potassium iodide, 5 grain tablets.
2. 5% acetic acid.

The Standard Lovibond Comparator Discs 3/2 I, 3/2 ARP and 3/2 APH

3/2 APH.	2.0 to 10.0	parts per million chlorine (Cl)
3/2 ARP.	5.0 to 50.0	„ „ „ „
3/2 I.	5.0 to 250	„ „ „ „

Disc 3/2 APH must be used with the Special-Purposes Comparator and a 40 mm. cell.

Technique

Fill both test vessels with the water to be tested and add the reagent to that in the right-hand compartment, so that the left-hand vessel contains a " blank " of the water behind the colour standard, to act as compensation for any inherent colour. Hold the comparator facing a uniform source of white light — a north light when possible is best — and rotate the disc until the colour of the liquid is matched by one of the glass standards. The value, expressed in parts per million, is then read from the indicator window.

***Discs* 3/2 I, 3/2 ARP.** Fill the test tubes to the 10 ml. mark, add 1 potassium iodide tablet to the right-hand tube, mix and match at once. Should the water be alkaline, add 0.5 ml. of 5% acetic acid solution to render the water faintly acid. This is not critical, so long as the solution is acid to litmus paper. Nitrites could interfere if the *p*H is below 6.0, but the use of chlorine in such quantity as to give residuals falling within the range of these discs would oxidise any nitrites originally present, so that in practice such interference is unlikely to arise.

***Disc* 3/2 APH.** To 50 ml. sample add 1 ml. of 5% acetic acid, or enough to render faintly acid to litmus paper, and 1 tablet of potassium iodide. Mix and transfer to a 40 mm. cell in the Special-Purposes Lovibond Comparator. Match at once.

The Determination of Residual Chlorine (5)

using the A.P.H.A. acid *o*-tolidine method.

Introduction

The *ortho*-tolidine test is widely used for the routine determination of residual chlorine in the control of water treatment. Chamberlin and Glass[1] have demonstrated that, for correct colour development, the reaction between chlorine and *o*-tolidine must be carried out at a *p*H of 1.3, or lower, and the ratio of *o*-tolidine to chlorine must be at least 3:1. These workers have suggested modifications to the original acid *o*-tolidine procedure, and their method has been accepted as a standard procedure by the American Public Health Association[2] and by other authorities.

Principle of the method

In acid solution chlorine reacts with *o*-tolidine giving a yellow holoquinone colour. The intensity of this colour is proportional to the chlorine concentration, which is determined by comparing the colour with a series of Lovibond permanent glass standards.

This method measures both free and combined chlorine. If it is required to estimate the free and combined chlorine separately, an approximate differentiation may be obtained by using Hallinan's *o*-tolidine arsenite (OTA) method[3]. This also provides a correction for interfering substances.

Where for any reason a more accurate estimation of the concentrations of free and combined chlorine is required, one of the alternative methods developed by Palin [4, 5, 6] should be used.

Reagents required

1. A.P.H.A. *o*-tolidine reagent

o-tolidine, analytical reagent grade	1 g.*
Hydrochloric acid, analytical reagent grade	150 ml.
Redistilled water, zero chlorine demand, to produce	1000 ml.

*Alternatively *o*-tolidine dihydrochloride may be used, in which case 1.35g. per litre of solution should be added.

2. Sodium arsenite solution

Sodium arsenite, analytical reagent grade,	5 g.
Redistilled water, zero chlorine demand, to produce	1000 ml.

Caution. Sodium Arsenite is TOXIC. TAKE CARE TO AVOID INGESTION.

The Standard Lovibond Comparator Disc 3/47

This disc covers the range 0.1 to 1.0 p.p.m. of chlorine. The colours of the 9 standards in this disc exactly match the modified Scott chromate-dichromate colour solutions as specified in the A.P.H.A. Standards[2]. The calibration of the disc has been checked and approved by Dr. A. T. Palin.

Technique

Total residual chlorine

Fill a 10 ml. comparator cell or test tube with the water to be tested. Place this filled cell in the left-hand compartment of the comparator to provide a blank and thus compensate for any inherent colour in the sample. To an identical cell or test tube add 0.5 ml. of the *o*-tolidine solution (reagent 1), fill to the mark with sample, mix, and place in the right-hand compartment of the comparator.

Hold the comparator facing a uniform source of white light, north daylight wherever possible, and rotate the disc until the colour of the liquid is matched by one of the Lovibond permanent glass standards. Matching should be carried out at the time of maximum colour development, but in any case no longer than 5 minutes after adding the reagent.

The concentration of chlorine in the sample, expressed in p.p.m. is then read from the indicator window.

OTA method for free and combined chlorine

To a 10 ml. cell or test tube add 0.5 ml. of *o*-tolidine solution (reagent 1), fill to the mark with sample and mix quickly. Immediately (within 5 seconds) add 0.5 ml. of arsenite solution (reagent 2), mix quickly and match against the colour standards as before. Record this value as reading **A.**

To a second cell or test tube add 0.5 ml. of arsenite solution (reagent 2), fill to the mark with sample and mix quickly. **Immediately** add 0.5 ml. of *o*-tolidine solution (reagent 1), mix quickly and match against the colour standards as quickly as possible. Record this value as reading **B.** Match again in exactly 5 minutes. Record this value as reading **C.**

To a third cell or test tube add 0.5 ml. of *o*-tolidine solution (reagent 1) and fill to the mark with sample. Mix quickly and match against the standards in exactly 5 minutes. Record this value as reading **D.** This represents the total amount of residual chlorine present plus the total amount of interfering colours.

The individual components can now be calculated as follows :—

Total residual chlorine	=	D — C
Free available chlorine	=	A — B
Combined available chlorine	=	Total minus Free
	=	B + D — (A + C).

Note

If it is only necessary to ensure that the level of the residual chlorine falls within the recommended limits it is possible to use a simplified procedure. Details of a special comparator, designed for the limit testing of both chlorine and *p*H in water, may be obtained from Tintometer Ltd., Salisbury, England.

References

1. N. S. Chamberlin and J. R. Glass, *J. Am. Water Works Assoc.*, 1943, **35,** 1065
2. "*Standard Methods for the Examination of Water and Waste Water,*" 11th Edition, American Public Health Association, New York, 1960
3. F. J. Hallinan, *J. Am. Water Works Assoc.* 1944, **36,** 296
4. A. T. Palin, *J. Inst. Water Eng.*, 1949, **3,** 100
5. A. T. Palin, *Analyst,* 1945, **70,** 203
6. "*Colorimetric Chemical Analytical Methods,*" 6th Edition, The Tintometer Ltd., Salisbury, England, 1961

The Determination of Chlorine Dioxide

using diethyl-*p*-phenylene diamine (Palin DPD)

Introduction

Chlorine dioxide is being increasingly used, especially in America, for the sterilization of water for domestic use. It is also used to render drinking water more palatable by removing unpleasant tastes.

The present test is designed for the estimation of residual traces of chlorine dioxide in water which has been treated with this agent. It is a modification[1,2] of the well-known Palin DPD method[3,4,5,6,7] for the determination of residual chlorine in water. As with the original method the present test may be adapted to the differential determination of residual chlorine compounds. The simplicity of the original test, arising from the use of reagents compressed into tablet form, has been retained in the present procedure.

Principle of the method

The hydrolysis of chlorine dioxide in water is very complex and the amount of the available chlorine which reacts with other indicators, such as *ortho*-tolidine, is variable, being dependent on factors including the *p*H of the water, the degree of exposure to sunlight and the nature of any other soluble impurities which may be present. As only approximately one fifth of the available chlorine from chlorine dioxide is recovered by previous colorimetric methods, corresponding to the amount converted to chlorite ion, these methods are insensitive as well as variable.

It has been discovered[1,2] that if the sample is first acidified, in the presence of potassium iodide, and then rendered approximately neutral, by adding sodium bicarbonate, the chlorine may be recovered in full. The liberated chlorine may then be estimated by means of diethyl-*p*-phenylene diamine in the usual way[4,5,6] using EDTA to suppress interfering ions. The red colour produced in this reaction is stable and its intensity, which is proportional to the concentration of chlorine dioxide, is determined by comparison with Lovibond permanent glass standards.

Reagents required

1. DPD tablets Nos. 1, 2 and 3
2. Sulphuric acid (5% v/v solution of analytical reagent quality acid)
3. DPD Neutralising tablets

The Standard Lovibond Comparator Discs 3/40A and 3/40B

3/40A covers the range 0·1 to 1·0 p.p.m. of chlorine (Cl_2).

3/40B covers the range 0·2 to 4·0 p.p.m. of chlorine (Cl_2).

Both of these discs are calibrated for use with 13·5 mm. cells or test-tubes and must be used with the dulling screen provided. The same screen is used for both discs.

The chlorine values obtained by the use of these discs are identical with those obtained by the FAS titration[8]. The master discs, against which all reproductions are checked, were tested and approved by Dr. Palin.

Technique

(a) ***Chlorine Dioxide.*** Method for use when only chlorine dioxide is present.

Place a DPD No. 1 tablet and one DPD neurtalising tablet in a 13.5 mm. cell or test tube. To 10 ml. of sample in a separate tube add 0.1 ml. of dilute sulphuric acid (reagent 2) and one DPD No. 3 tablet. Mix vigorously. After about one minute add a few drops of the treated

sample to the cell containing the No. 1 tablet and the neutralising tablet and allow the tablets to disintegrate. Then add the remainder of the acidified sample and mix rapidly. Place the cell in the right-hand compartment of the comparator and place an identical cell filled with untreated samle in the left-hand compartment. Make sure that the dulling screen is in position and match the colour of the treated sample with the Lovibond permanent glass standards in the disc, using north daylight wherever possible. The figure then shown in the indicator window represents parts per million of available chlorine present as chlorine dioxide.

(b) ***The Differential Determination of Free Chlorine, Combined Chlorine (Chloramines) and Chlorine Dioxide.*** Method for use when the source of the residual chlorine is uncertain.

Proceed as in the determination of chlorine dioxide (a) above). Owing to the presence of free and combined chlorine the figure obtained now represents the *total residual chlorine content* of the sample.

Rinse a second 13·5 mm. cell, or test tube, with the sample and leave just enough of the sample in the tube to cover one DPD tablet. (If the monochloramine content proves to be high, differentiation may be improved by using distilled water rather than sample for disintegrating the tablet). Drop one DPD No. 1 tablet into the prepared cell and allow to disintegrate. Fill the cell to the 10 ml. mark with sample, mix rapidly and place the cell in the right-hand compartment of the comparator. Place an identical cell filled with untreated sample in the left-hand compartment and rapidly match the colour of the treated sample with the standards as before. The reading obtained is that of the free chlorine plus one fifth of the chlorine from chlorine dioxide. Call this *Reading No.* 1.

Add one DPD No. 2 tablet to the sample cell, mix vigorously, replace in the right-hand compartment of the comparator and immediately match with the standards in the disc. Call this *Reading No.* 2.

Finally add one DPD No. 3 tablet to the sample cell, mix vigorously, replace in the right-hand compartment of the comparator and match after two minutes. Call this *Reading No.* 3.

The chlorine concentrations of the individual components may then be calculated in the following manner.

Chlorine dioxide = (total residual chlorine — Reading No. 3) $\times \frac{5}{4}$

Free chlorine = Reading No. 1 — $\frac{1}{5}$ ClO_2.

Monochloramine = Reading No. 2 — Reading No. 1.

Dichloramine = Reading No. 3 — Reading No. 2.

Notes

1. If differentiation of the combined residual chlorine into mono- and di-chloramine fractions is not required it is unnecessary to use the DPD No. 2 tablet. This step in the procedure may therefore be omitted and the subsequent calculations modified accordingly.

2. In systems where only chlorine and chlorine dioxide are likely to be present, only the first part of the normal DPD method need be carried out (i.e. Reading 1 of *b* above). This gives the concentration of free chlorine together with one fifth of the chlorine dioxide. The acid modification (*a* above) then gives the concentration of free chlorine and the whole of the chlorine dioxide. From these two results the individual concentrations of free chlorine and of chlorine dioxide may readily be calculated.

3. Interference by copper ions (up to approximately 10 p.p.m. Cu) is normally prevented by chelation of the copper by the EDTA present in the DPD tablets. In the acid modification traces of copper may still give a colour when the reagent is added, owing to the potassium iodide being first used in acid solution. It is therefore recommended that after adding the acidified sample to the prepared cell, containing one DPD No. 1 tablet plus sodium bicarbonate solution, an interval of two minutes should elapse, followed by a final mix, before the colour is measured in the comparator. This will allow sufficient time for the solution of the EDTA and for the subsequent chelation of the copper.

4. Dissolved oxygen in the water can produce a faint colour with the reagent if allowed to stand. In the absence of trace-metal catalysis, which is suppressed by the EDTA present in the tablets, no interference should occur within the time of the test.

5. The presence of manganic ions in the water could interfere with the test. The interference may, however, be overcome by developing the manganese colour in the blank also, as follows :—

To 10 ml. of sample in a separate test tube add one DPD No. 2 tablet and one drop of 0·5% sodium arsenite ($NaAsO_2$) solution. Mix until dissolved. Rinse a comparator cell with sample, and add one DPD No. 1 tablet. Allow to disintegrate and then add the 10 ml. of arsenite treated sample. Mix and place in the left-hand compartment of the comparator.

6. The quantity of reagent used in the tablets has been chosen to suit the range of chlorine concentrations covered by the discs, *i.e.* up to 4 p.p.m. Samples containing higher concentrations must be diluted. If there is any doubt about the need for dilution a simple check is to repeat the procedure using two DPD No. 1 tablets instead of one. A marked increase in colour intensity indicates that dilution is required. In this case the required amount of distilled water is added to the reagents and the volume made up with sample. When a sample has been diluted the indicated chlorine content must be multiplied by the dilution factor.

7. As only a trace of potassium iodide is needed to develop a colour with chloramines in this test, all glassware must be thoroughly cleaned after making each test. Similarly care must be taken not to touch the tablets, particularly DPD No. 1 tablet, with the fingers.

References

1. A. T. Palin, *Proc. Soc. Water Treatment and Exam.*, 1960, **9**, 81
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4. A. T. Palin, *Analyst*, 1945, **70**, 203
5. A. T. Palin, *Baths Service*, 1958, **17**, 21
6. A. T. Palin, *Water and Water Eng.*, 1958, **62**, 30
7. *"Colorimetric Chemical Analytical Methods"*, The Tintometer Ltd., Salisbury, England, 1959
8. *"Standard Methods for the Examination of Water, Sewage and Industrial Wastes"*, 10th Edition, American Pub. Health Assocn., 1955

The Determination of Chromium

using diphenylcarbazide

Introduction

The following method was devised by B. Bagshawe for the estimation of chromium in steel, using the Lovibond Tintometer, and is reprinted from the *Journal of the Society of Chemical Industry* (see reference). The same method, however, with suitable modifications, is applicable to the analysis of other materials and solutions.

Principle of the method

The test is based on the colour reaction produced by diphenylcarbazide with dilute acidified chromate solutions.

The reagent also gives colour reactions with mercury, cadmium, magnesium, iron, copper, nickel, molybdenum and vanadium, but of these the first three are never found in steel, and iron, copper, and nickel are removed by the peroxidation treatment under the actual conditions obtaining in the test. Vanadium and molybdenum pass into the alkaline filtrate as soluble molybdates and vanadates together with chromium, and impart their characteristic colours on treatment with diphenylcarbazide. Thus the method is not applicable in the presence of vanadium and molybdenum.

Reagents required

1. Dissolve 1 g. of diphenylcarbazide in 100 ml. of 90% alcohol and acidify with 3 drops of sulphuric acid (*d* 1.84). The solution so obtained darkens considerably on prolonged keeping, and should be freshly made every two or three days.
2. Sulphuric acid, 15% by volume
3. Potassium permanganate solution (1% w/v)
4. Caustic soda solution 25% w/v
5. Sodium peroxide

Permanent standards

This test is carried out in the Lovibond Tintometer, and calibration curves are prepared by the operator to cover the appropriate range for which the test is required. The colour is matched by a combination of Lovibond red and blue glasses, but only the red value is plotted and considered significant for the purposes of this test. By choosing the appropriate fraction from the two prepared, and by varying the depth of cell employed, it is possible to cover a very wide range of concentrations. For example, using a 6″ depth, the author quoted Lovibond values for chromium from .001 to .015 per cent. and using a 1″ cell the range of chromium from .01 to 0.1 per cent. He stated that the upper limit of applicability of the test is 0.2% chromium.

Technique

The following method is reprinted from the article quoted, and refers to steel analysis, but suitable modifications may be made for other circumstances.

A 1 g. sample of steel is weighed into a 400 ml. flask and dissolved with 30 ml. of 15% sulphuric acid (by vol.). When dissolution is complete the solution is oxidised with the minimum amount of concentrated nitric acid and digested until red nitrous fumes are completely expelled. The solution is now diluted to approximately 100 ml. with water, raised to boiling, and treated with a few drops of dilute potassium permanganate solution (10 g. per litre) until the pink colour persists after boiling for 2 minutes. The solution is now allowed to cool slightly and is then treated with 25 ml. of caustic soda solution (250 g. per litre), followed by 1 g. of sodium peroxide. After these additions the solution is reheated to boiling and maintained at a gentle boil for 3 to 4 minutes. Care is necessary at this

point as some solutions exhibit a marked tendency to bump. The solution together with the precipitated ferric hydroxide is made up to exactly 500 ml. in a graduated flask with cold water, mixed, and two separate aliquot parts, one of 50 ml. and one of 100 ml., obtained by filtration through a dry fluted paper. The two fractions are transferred to separate 250 ml. graduated flasks, and each is treated in turn with 50 ml. of 15% sulphuric acid (by vol.) and 2 ml. of the diphenylcarbazide solution, made up to the mark, and mixed. With a little experience the colour of the two solutions at this point should furnish a guide to the more applicable of the two solutions, with regard to which is of a suitable intensity for colour comparison and also in what depth the sample should be viewed. Comparison is then made against similarly prepared standards containing known amounts of chromium.

Notes

1. The violet colour produced in the reaction fades slowly, but is sufficiently stable for determination in a reasonable time.

2. In the article quoted, sample calibration curves are given, and very satisfactory accuracy shown by figures of actual tests performed on known samples.

Reference

B. Bagshawe, *J.S.C.I.*, 1938, LVII, 260

The Determination of Chromium (2)

using diphenylcarbazide

Introduction

This method was originally developed[1] for the determination of chromate and total chromium in sewage and sewage effluents. However, provided that a suitable form of pre-treatment is used to extract the chromium, and to destroy any organic matter which is present in the sample, this method can be applied to a wide variety of samples[2].

Principle of the method

Hexavalent chromium, in acid solution, reacts with diphenylcarbazide to give a red-violet colour the intensity of which is proportional to the concentration of hexavalent chromium present in the solution. This colour is compared with a series of Lovibond permanent glass standards. By analysing the sample for chromium before and after oxidation, the proportion of both chromate and of total chromium may be determined.

Reagents required

1. Nitric acid, concentrated
2. Perchloric acid, 60 per cent
3. Hydrochloric acid, 5N
4. Diphenylcarbazide solution, 0.2g. diphenylcarbazide in 100 ml. ethyl alcohol. This solution must be kept as cool as possible and should be dicarded immediately it becomes brown in colour.
5. Sulphuric acid, cautiously add 1 volume of the concentrated acid to one volume of distilled water
6. Silver nitrate solution 5g. silver nitrate in 100 ml. distilled water
7. Sodium nitrite solution 1g. sodium nitrite in 100 ml. distilled water
8. Ammonium persulphate
9. Phosphoric acid sp. gr. 1.75

All chemicals used should be of analytical reagent quality.

The Standard Lovibond Discs, Comparator Disc 3/59 and Nessleriser Disc NOK

Disc 3/59 covers the range 10-100 μg. of chromium (Cr) in 50 ml. of solution, i.e. 0.2 to 2 p.p.m.

Disc NOK covers the range 2-10 μg. of chromium (Cr) in 50 ml. of solution, i.e. 0.04 to 0.2 p.p.m.

A dulling screen must be used with both discs.

Technique

(a) Preliminary treatment of sample. *Unless the sample is already in solution, and it is certain that organic matter is not present*, place a suitable amount of sample (an amount thought to contain either 10 or 100 μg. of chromium, depending on whether a Comparator or a Nessleriser is being used, is a suitable quantity) in a beaker, add 5 ml. of concentrated nitric acid (reagent 1), and evaporate to a small volume. Transfer the concentrated solution to a porcelain or silica basin and, if necessary, further reduce the volume to about 15 ml. on a water-bath. Add 2 ml. of perchloric acid (reagent 2) and evaporate to dryness. Remove the source of heat as soon as the sample becomes dry. Transfer to a furnace and ignite at just below 300°C. for about 40 minutes. Dissolve the residue by adding 2 ml. of hydrochloric acid (reagent 3) and a little distilled water. Warm gently. If the solution is clear, with neither turbidity nor a precipitate, transfer to a volumetric flask and make up to 50 ml. with distilled water. If the solution is not clear and has a brownish colour this indicates that the oxidation of organic matter is still incomplete, and the processes of evaporation to dryness, followed by ignition and solution, should be repeated until a colourless product is obtained.

If a white precipitate or turbidity is obtained at this stage remove by filtration through a sintered glass filter (porosity 4) and continue the analysis on the clear filtrate, which should be made up to 50 ml. as above.

(b) Total chromium. To 25 ml. of the solution of the sample add 5 ml. of dilute sulphuric acid (reagent 5). Remove any chloride ions which may be present by evaporating until white fumes appear. Cool and dilute to about 30 ml. with distilled water. Bring solution nearly to the boiling point and then add 1 ml. of silver nitrate solution (reagent 6), followed by about 1 g. of ammonium persulphate (reagent 8). Boil for at least 10 minutes. If the solution at this stage has a pink tinge, due to the presence of manganese (see Note 2), add sodium nitrite solution (reagent 7) drop by drop until the colour just disappears. Care must be taken not to add too much nitrite solution as excess nitrite will reduce the chromate and lead to low results. Transfer the solution to a volumetric flask, add 1 ml. phosphoric acid (reagent 9) and make up to 50 ml. with distilled water. Mix, add 2 ml. diphenylcarbazide solution (reagent 4) mix again and allow to stand for 5 minutes to ensure full colour development. Transfer to a Nessleriser tube and place in the right-hand compartment of the Nessleriser. Prepare a blank on the reagents by carrying out all the manipulations described above, including pre-treatment where this has been used, using distilled water in the place of the sample as starting material. Place a Nessleriser tube filled with this blank in the left-hand compartment of the Nessleriser and compare the colour of the sample with the Lovibond permanent glass standards in the disc using north daylight wherever possible.

If the chromium concentration is higher than the highest Nessleriser standard either repeat the colour measurement using a comparator with the appropriate disc rather than the Nessleriser, or alternatively, repeat the determination using a smaller aliquot of the sample.

(c) Chromium present as chromate. Take an aliquot of the original sample before pre-treatment, filter if necessary through sintered glass, add 5 ml. of sulphuric acid (reagent 5) and dilute to 50 ml. with distilled water. Add 1 ml. phosphoric acid (reagent 9), mix, add 2 ml. of diphenylcarbazide solution (reagent 4), mix again and allow to stand for 5 minutes. Prepare a blank using distilled water in the place of the sample. Compare the colour of the sample with the colours of the Lovibond permanent glass standards in the appropriate disc as in (**b**) above.

Notes

1. Iron interferes with the determination of chromium by giving a yellow to brown coloration. If the ratio of iron to chromium does not exceed 100 : 1 the interference can be suppressed by the addition of phosphoric acid. If the proportion of iron is too high for suppression to be effective then the iron must be removed from the sample, by precipitation as ferric hydroxide, before the chromium is determined.

2. In the method for total chromium, manganese interferes if it is present in amounts greater than 0.2 p.p.m. The presence of manganese is indicated by the appearance of a pink colour during the chromium determination, and the interference can be removed by discharging the pink colour by titration with sodium nitrite.

3. The figures on the discs represent the concentration of chromium in the final solution. The chromium content of the original sample can be obtained from this by simple proportion.

4. The readings obtained by means of the B.D.H. Lovibond Nessleriser and disc are accurate only when the Nessleriser glasses used conform to the specification employed when the disc was calibrated; that is that the 50 ml. calibration mark shall fall at a height of 113 ± 3mm. measured internally.

References

1. Ministry of Housing and Local Government, "*Methods of Chemical Analysis as applied to Sewage and Sewage Effluents.*" H.M.S.O., London, 1956
2. E. B. Sandell, "*Colorimetric Determination of Traces of Metals.*" Interscience Publishers Inc., New York, 1950

The Determination of Cobalt (1)

using thiocyanate (Vogel's reaction)

Introduction

Apart from its use in a wide variety of industries, from metallurgy to its use as an accelerator in the oxidation of certain oils, cobalt appears to be present in small amounts in many naturally occurring materials. The estimation of cobalt is therefore of interest to chemists in a variety of fields.

Principle of the method

This test is based on the reaction between cobalt ions and concentrated thiocyanate solutions to give blue cobalt thiocyanate ions which are soluble in amyl alcohol.

Reagents required

1. Hydrochloric acid 5N
2. Potassium permanganate 0.1N
3. Ammonium thiocyanate 7.5M. aqueous solution
4. A mixture of equal volumes of amyl acetate and amyl alcohol
5. Ammonium phosphate $(NH_4)_2\ HPO_4$ approximately M. aqueous solution

The Standard Lovibond Comparator Disc 3/31

The disc covers the range 10γ to 200γ (0.01 to 0.2 mg.) of cobalt (Co).
A dulling screen must be used with this disc.

Technique

Take a weighed quantity of the sample, up to 5 g., dissolve in 10 ml. of water, add 1 ml. of 5N-hydrochloric acid and 1 drop of 0.1N-potassium permanganate: after mixing, add 5 ml. of an approximately 7.5 M. aqueous solution of ammonium thiocyanate. Extract the mixture with 3 separate 3.3 ml. portions of solvent, comprising equal volumes of amyl acetate and amyl alcohol. After these 3 extractions, discard the aqueous layer and combine the solvent layers together. Shake with a mixture of 5 ml. of an approximately M. aqueous solution of ammonium phosphate, $(NH_4)_2\ HPO_4$, to prevent interference from any ferric iron present, and 5 ml. of 7.5 M. aqueous solution of ammonium thiocyanate. After separation, reject the lower layer and wash the solvent layer once with a mixture consisting of 1 ml. of M-ammonium phosphate, 2 ml. of 7.5 M-ammonium thiocyanate and 1 ml. of water. Draw off the lower layer. The colour of the upper layer of amyl acetate-amyl alcohol is then compared in a 13.5 mm. test tube or cell with the standard disc in the comparator, held facing a good north light.

The figures represent micrograms of Cobalt in the weight of sample taken.

Reference

" AnalaR Standards for Laboratory Chemicals," 5th Edition, 1957,
(British Drug Houses Ltd. and Hopkins & Williams Ltd., England.)

The Determination of Cobalt (2)

using nitroso-R salt

Introduction

The presence of trace amounts of cobalt in foodstuffs, natural or manufactured, has been shown to be essential to the well being of sheep and cattle. The examination of these foodstuffs for cobalt has consequently become important. This test has been developed with this application in mind and is recommended for the determination of cobalt in biological materials. The method can, however, be applied for other purposes in the absence of heavy metals and iron. (See Note 1).

Principle of the method

The test is based on the colours produced by the reaction, under specified conditions, between cobalt and nitroso-R salt (sodium 1-nitroso-2-hydroxy-naphthalene-3 : 6-disulphonate). This reagent was originally proposed as a qualitative test for cobalt by H. S. van Klooster[1] while F. J. Stare and C. A. Elvehjem[2] suggested its application for the colorimetric determination of cobalt in biological materials. Several modifications of this quantitative method have been described and the procedure given here is essentially that due to K. J. McNaught[3].

The preliminary procedure will vary according to the character of the sample under investigation. Organic material is best submitted to wet-oxidation using sulphuric acid and nitric acid, and after the complete removal of the latter, the remaining concentrated sulphuric acid is diluted with water and the solution examined for cobalt. The sample which is to be tested should always be in the form of a solution in diluted hydrochloric acid or diluted sulphuric acid.

Reagents required

1. Sodium acetate (hydrated) (analytical reagent grade)
2. A 0.2 per cent w/v alcoholic solution of phenolpthalein (analytical reagent grade)
3. Sodium hydroxide approximately 5N
4. Sulphuric acid approximately N and sulphuric acid approximately N/20
5. A 0.1 per cent. w/v aqueous solution of nitroso-R salt which should be freshly prepared each day.
6. Nitric acid sp. gr. 1.42 (analytical reagent grade)

The Standard B.D.H. Lovibond Nessleriser Discs NTA and NTB

Disc NTA covers the range 1γ to 9γ (0.001 to 0.009 mg.) of cobalt (Co).

Disc NTB covers the range 10γ to 30γ (0.01 to 0.03 mg.) of cobalt (Co).

Disc NTB requires the use of a dulling screen.

Technique

To 5 ml. of the solution under examination add 2g. of sodium acetate and a few drops of phenolphthalein solution, warm the mixture on a boiling water-bath and render faintly alkaline with 5N sodium hydroxide; then add, first suphuric acid approximately N/1 and finally sulphuric acid approximately N/20 until the pink colour of the indicator almost disappears (approximately *p*H 8.3). Add 1 ml. of the solution of nitroso-R salt, boil gently for one minute and, while still boiling, add slowly 2 ml. of nitric acid. Continue the boiling for about a minute, then cool the mixture under the tap, transfer it to a Nessleriser glass, dilute with water to the 50 ml. mark and place in the right-hand compartment of the Nessleriser. Conduct a control test starting with 5 ml. of normal sulphuric acid and employing the same reagents and procedure as above and transfer the final mixture to a Nessleriser glass, dilute with water to the 50 ml. mark and place in the left-hand compartment of the instrument.

Stand the Nessleriser before a uniform source of white light — a north window wherever

possible is the best — and compare the colour produced in the test solution with the colours in the standard disc, rotating the disc until a colour match is obtained.

The markings on the disc represent the actual amounts of cobalt (Co) producing the colours in the test. If a colour equivalent to 10γ (0.01 mg.) is produced in the test, then the quantity of solution taken for the test contains 10γ (0.01 mg.) of Co.

If the colour obtained is deeper than the standard colours, the test should be repeated on a smaller quantity of the solution.

Notes

1. The test is applicable in the presence of nickel but heavy metals or iron interfere. The former can be removed from an acid solution with hydrogen sulphide and the latter may be extracted with ether from a solution which is 5N with respect to hydrochloric acid. N. D. Sylvester and L. H. Lampitt (*J. Soc. Chem. Ind.*, 1940, **59**, 57) describe a method for the separation of cobalt from the ash of food-stuffs which involves precipitating the cobalt, together with any iron and copper that may be present, with 1-nitroso-2-naphthol, followed by a wet-oxidation of the precipitate and extraction under specified conditions with diphenylthiocarbazone. After wet-oxidation of the second residue the cobalt is determined colorimetrically with Nitroso-R salt. This procedure eliminates any possible disturbance due to the presence of a relatively large quantity of calcium. The original paper should be consulted for the working details appertaining to the isolation of the cobalt, but for the final colorimetric determination by means of the Nessleriser the method given here should be followed.

2. B.D.H. Lovibond Nessleriser discs are standardised on a depth of liquid of 113 $\pm$ 3 mm. The height, measured internally, of the 50 ml. calibration mark on Nessleriser glasses used with the instrument must be within the same limits. Tests with Nessleriser glasses not conforming to this specification will give inaccurate results.

References

1. H. S. van Klooster, *J. Amer. Chem. Soc.*, 1921, **43**, 746
2. F. J. Stare and C. A. Elvehjem, *J. Biol. Chem.*, 1932, **99**, 473
3. K. J. MacNaught, *Analyst*, 1939, **64**, 23

The Determination of Copper (1)

using zinc dibenzyldithiocarbamate

Introduction

This method is useful because copper may be determined on large volumes of material by direct extraction after making the sample solution Normal to sulphuric acid. Also the necessity of adding a complexing agent and of rendering the solution alkaline with ammonia, as for the diethyldithiocarbamate method, is obviated.

Principle of the method

Zinc dibenzyldithiocarbamate combines with copper forming a mixture of zinc and copper dithiocarbamate. On making this mixture, in carbon tetrachloride, acid by means of dilute sulphuric acid, the zinc is transferred to the aqueous phase while the copper dithiocarbamate remains in the carbon tetrachloride.

Reagent required

0.05 g. zinc dibenzyldithiocarbamate dissolved in 100 g. carbon tetrachloride (analytical reagent grade).

The Standard Lovibond Comparator Disc 3/39

The disc covers the range 2.5γ to 50γ (0.0025 to 0.05 mg.) of copper (Cu.).

Technique

Take a suitable volume of the sample, which contains enough copper to bring it within the range of the disc, and make it approximately Normal in respect to sulphuric acid. Add 10 ml. of reagent and shake vigorously in a separating funnel for 30 seconds. Separate the yellow carbon tetrachloride layer and match in a 13.5 mm. cell in the Lovibond Comparator against the standard colour disc.

The answer represents micrograms copper in the amount taken.

A blank test must be performed on the amount of sulphuric acid used to acidify the sample, and any copper found deducted from the above answer.

Notes

1. The disc may also be used for the determination of copper in malt beverages by a non-ashing technique. (I. Stone, *et al.*, *Anal. Chem.* 1953, **25,** 893). Also for the estimation of copper in cyder, (C. F. Timberlake. *Chem. and Ind.* 1954, **47,** 1442) and in oils and fats, (D. C. Abbot *et al.*, *Analyst*, 1954, **79,** 547).

2. The following metals interfere; bismuth, nickel and cobalt, which form yellow complexes with the reagent; mercury (II), silver and antimony hinder the extraction of the copper complex. Further details on this point are given in " Organic Reagents for Metals," Volume I, published by Messrs. Hopkin & Williams Limited, London, page 188 *et seq.*

Reference

R. I. Martens and R. E. Githens, *Anal. Chem.*, 1952, **24,** 991

The Determination of Copper (2)

using dithio-oxamide (rubeanic acid)

Introduction

The following method is useful in those cases where the presence of chromium and manganese complicate the procedure for the diethyldithiocarbamate test.

Principle of the method

The test is based on the colours produced by the action of dithio-oxamide (rubeanic acid) on copper. Dithio-oxamide also yields colours with nickel and cobalt but in solutions containing free acetic acid the sensitivity of the reactions with those metals is greatly depressed although the delicate response to copper persists. Colours or precipitates are also produced with platinum, palladium and silver, while iron, if present in more than a faint trace, gives rise to a brown tint and interferes with the determination of copper. Traces of mercury and most other heavy metals tend to prevent the full colour development of the colour due to copper, but small amounts of aluminium, bismuth, chromium, calcium, manganese and the alkali metals do not interfere. As the colour is due to the formation of a compound in a colloidal state, the solution to be tested should be as free as possible from electrolytes.

Reagents required

1. Acetic acid, approx. 5N
2. A 20% w/v aqueous solution of ammonium acetate (analytical reagent grade).
3. A 1% w/v aqueous solution of gum acacia prepared by dissolving 10 grammes of gum acacia in 1 litre of water, boiling to destroy oxidases, filtering and preserving with thymol.
4. A 0.2% w/v solution of dithio-oxamide in 90% alcohol

The Standard B.D.H. Lovibond Nessleriser Discs NDA, NDB and NDAB

Disc NDA covers the range 2.5γ to 40γ (0.0025 to 0.04 mg.) of copper (Cu.).
Disc NDB covers the range 30γ to 100γ (0.03 to 0.1 mg.) of copper (Cu.).
Disc NDAB covers the range 10γ to 100γ (0.01 to 0.1 mg.) of copper (Cu.).

Technique

To a suitable quantity of the solution under examination (which should be approximately neutral) contained in a Nessleriser glass, add 1 ml. of 5N acetic acid, 5 ml. of ammonium acetate solution and 1 ml. of gum acacia solution ; dilute with distilled water to 50 ml. and add 0.5 ml. of the dithio-oxamide solution. Mix and allow to stand for five minutes, for the colour to develop, and then place in the right-hand compartment of the Nessleriser. In another Nessleriser glass place 1 ml. of 5N acetic acid, 5 ml. of ammonium acetate solution, and 1 ml. of gum acacia solution ; dilute with distilled water to 50 ml. and add 0.5 ml. of the dithio-oxamide solution. Mix and place in the left-hand compartment of the Nessleriser. Stand the Nessleriser before a uniform source of white light — a north window is the best — and compare the colour produced in the test solution with the colours in the standard disc, rotating the disc until a colour match is obtained.

The markings on the disc represent the actual amount of copper (Cu.) producing the colours in the test. Should the colour in the test solution be deeper than the standard colour glasses, a fresh test should be conducted using a smaller quantity of the sample under examination. The standardization of the glasses has been carried out at temperatures between 20° and 25° C. and it is recommended that the same condition should be observed when using the disc.

Notes

1. Distilled water prepared in metal stills usually contains traces of copper and gives a distinct colour in this test. Distilled water (analytical reagent grade) or water re-distilled from glass should be used throughout the test.

2. It must be emphasized that the readings obtained by means of the B.D.H. Lovibond Nessleriser and disc are only accurate provided that Nessleriser glasses are used which conform to the specification employed when the discs were being calibrated, namely that the 50 ml. calibration mark shall fall at a height of 113 mm. plus or minus 3 mm. measured internally.

Reference

N. L. Allport and G. H. Skrimshire, *Quart. J. Pharm.*, 1932, **5**, 461

The Determination of Copper (3)

using sodium diethyldithiocarbamate

Introduction

In many processes in industries, especially those in any way connected with foodstuffs, cosmetics, rubber, public health, and metallurgy, it is frequently required to estimate small quantities of copper, usually present as an impurity.

Principle of the method

The delicate colour test for copper employing sodium diethyldithiocarbamate was suggested by Callan and Henderson.[1] These investigators showed, however that several other metals yield colours or precipitates with the reagent. The utility of the test was extended by Haddock and Evers[2] who advocated extraction of the coloured copper compound with carbon tetrachloride, thereby obviating the interference of most other substances. A modification of this method has been adopted for the purpose of standardising the comparator disc. The test is applicable in the presence of ferric iron up to 0.1 gram, but ferrous iron in quantities exceeding 1 mg. interferes. Alkali metals and small amounts of calcium, aluminium, zinc, nitrates, sulphites and phosphates are without influence, but special procedures are described by Haddock and Evers[2] for applying the test in the presence of chromium, manganese and tin.

Reagents required

1. A 20 per cent. w/v aqueous solution of citric acid, (analytical reagent grade).
2. Solution of Ammonia containing 10 per cent. w/v NH_3
3. A 0.1 per cent. aqueous solution of sodium diethyldithiocarbamate.
4. Carbon tetrachloride (analytical reagent grade).
5. Sodium sulphate anhydrous (analytical reagent grade).

The Standard Lovibond Comparator Disc 3/5

The disc covers the range 2.5γ to 50γ (0.0025 to 0.050 mg.) of copper (Cu.), which equals 0.25 to 5.0 parts per million if a 10 ml. sample is taken.

Technique

Transfer 10 ml. of the neutral solution under examination to a small separating funnel, add 10 ml. of the citric acid solution and 6 ml. of the dilute solution of ammonia ; mix and add 10 ml. of the sodium diethyldithiocarbamate solution, followed immediately by 2.5 ml. of carbon tetrachloride Shake vigorously, allow to separate and run the lower layer into a dry 10 ml. measure. Repeat the extraction with three more portions, each of 2.5 ml. of carbon tetrachloride and transfer them to the same 10 ml. measure. If necessary add more carbon tetrachloride to produce 10 ml., add about 0.5 g. of anhydrous sodium sulphate, shake and pour off the clear liquid into one of the comparator test tubes and place in the right-hand compartment of the comparator. At the same time carry out a blank determination using if possible 10 ml. of the water which was employed as a solvent for the sample under examination, and the same quantities of all the reagents, and place the tube containing the carbon tetrachloride extract of this in the left-hand compartment of the comparator. This is to compensate for the presence of any copper already present in this water. Hold the comparator facing a uniform source of white light—a north window wherever possible is the best—and compare the colour produced in the test solution with the colours in the standard disc, rotating the latter until a colour match is obtained. The amount of copper present in the test solution is then shown in the indicator recess in the right-hand bottom corner of the comparator.

Notes

If the colour produced in the test is deeper than the standard colours the test should be repeated using a smaller quantity of the original liquid under examination, previously diluted to 10 ml. with distilled water. Extremely minute traces of copper may be determined by evaporating an appropriate volume of the liquid under examination to 10 ml. and then applying the method as described above.

References

1. T. Callan and J. A. R. Henderson, *Analyst*, 1929, **54**, 650
2. L. A. Haddock and N. Evers, *Analyst*, 1932, **57**, 495

The Determination of Copper (4)

using sodium diethyldithiocarbamate

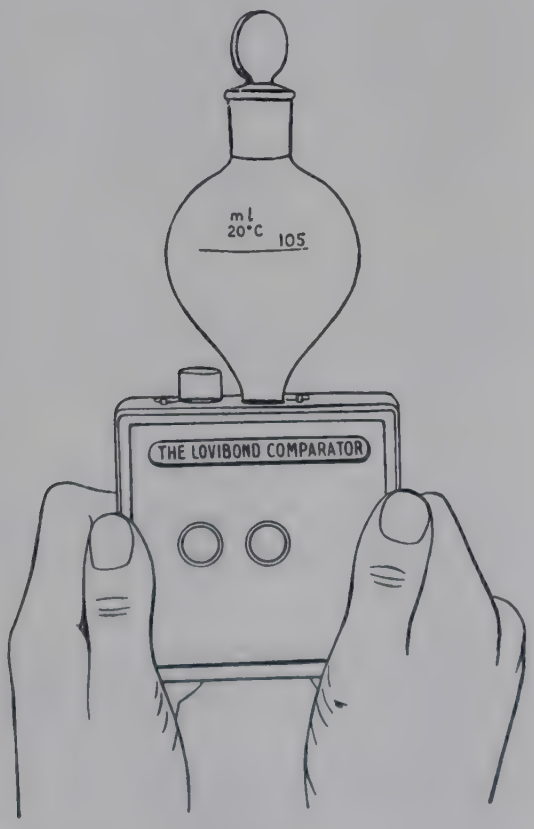
Introduction

Traces of copper in a water supply often give rise to trouble in galvanised cisterns and hot-water tanks. This does not necessarily mean that copper plumbing should not be mixed with galvanised tanks, as with some waters it does no harm, but many deep well waters are cupro-solvent, dissolving traces of metal from copper rising mains. These waters can themselves lessen the resistance to corrosion of galvanised steel, and, in the presence of copper, failure sometimes results in a short time. If copper is detected in the water, precautions should be taken to prevent such failures by protecting galvanised steel cisterns with bitumen paint, or a magnesium anode can be used in either hot-water tanks or cisterns.

The British Non-Ferrous Metals Research Association[4] has devised a simplified method for copper determination whereby these tests can easily be carried out on the site. There is thus no difficulty in testing for the presence of small traces of copper in any water supply.

Precautions to protect galvanised steel should generally be taken if the copper content of the water approaches 0.1 parts per million.

A complete kit, containing all the reagents and equipment required for carrying out the test, is available (Note 3).



Principle of the method

Sodium diethyldithiocarbamate forms yellow copper diethyldithiocarbamate when added to alkaline solutions containing traces of copper[1]. This coloured compound is soluble in organic solvents[2] and extraction with carbon tetrachloride separates the copper diethyldithiocarbamate from the diethyldithiocarbamates of other metals, such as iron, which would otherwise interfere with the determination. The intensity of the yellow colour of the carbon tetrachloride extract is proportional to the copper concentration, and is measured by comparison with Lovibond permanent glass standards.

Reagents required

1. **Mixed reagent:** The following parts, by weight, of reagents are ground together until the mixture is homogeneous, and stored in a brown bottle :—

15 parts ammonium chloride
15 parts sodium carbonate
30 parts ammonium citrate (copper free)
0.4 parts sodium diethyldithiocarbamate
} analytical reagent grade

2. Carbon tetrachloride (analytical reagent grade)

The Standard Lovibond Comparator Disc 3/5

This disc covers the range 2.5γ to 50γ (0.0025 to 0.050 mg.) of copper (Cu). This corresponds to 0.0125 to 0.25 p.p.m. on 100 ml. sample.

Technique

Into both the special extractor (See diagram) and the test tube measure 5 ml. of carbon tetrachloride. Measure into the extractor 100 ml. of the water to be tested. Add 2-3 g. (1 heaped capful) of the mixed reagent (reagent 1) to both the test tube and to the extractor using the funnel provided. Shake the extractor, to dissolve the reagent, and allow to stand for 5 minutes, To the test tube add pure copper-free water (preferably demineralised) to the 10 ml. mark, stopper, with a rubber bung covered with a small square of polythene (Note 1), shake, to dissolve the reagent, and allow to stand for 5 minutes.

After standing for the required time, shake both the extractor and the test tube for 3 minutes to extract the yellow colour into the carbon tetrachloride layer (Note 2). Place the test tube in the left-hand compartment of the Lovibond Comparator, and the extractor tube in the right-hand compartment. Match the colour in the extractor tube against the permanent glass standards in the disc, using north daylight wherever possible. Read off the copper concentration from the indicator window in the comparator, and convert the reading into parts per million of copper by means of the following table :—

Disc Reading	B.N.F. Kit corrected Cu concentration
γ	p.p.m.
2.5	0.0125
5.0	0.025
10.0	0.05
15.0	0.075
20.0	0.10
25.0	0.125
30.0	0.15
40.0	0.20
50.0	0.25

Notes

1. A fresh piece of polythene should be used for each determination.
2. "Bubbles" in the carbon tetrachloride layer may be cleared by gently rotating the tubes in their holders.
3. A special field kit containing :
   A Lovibond Comparator
   Disc (code 3/5) containing 9 permanent glass colour standards
   Glass extractor (100 ml. capacity) to fit comparator
   Calibrated test tube
   Polythene bottle of carbon tetrachloride
   Polythene wash bottle for distilled water
   Bottle of "mixed reagent" in powder form
   Rubber bung and a supply of small squares of polythene sheet
   Polythene funnel for pouring powder into tube and extractor
   Instructions for use
   may be obtained from The Tintometer Ltd., Salisbury, England.

References

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2. L. A. Haddock & N. Evers, *Analyst*, 1932, **57**, 495
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The Determination of Copper Sulphate

Introduction

The maintenance of an adequate strength of copper sulphate in copper-plating baths has always been recognised as of great importance, but direct determinations are seldom used for the purpose. Some workers use the indirect method of measuring the density by a hydrometer, while others use the empirical method of colour judgement by eye. It is obvious that such a test should be placed upon a more scientific basis, and the disc herein described has been prepared for this purpose.

Principle of the method

No reagent is used, the colour of the solution itself being an adequate indication when viewed in a suitable apparatus.

The Standard Lovibond Comparator Disc 3/16

This disc covers the range 125 grams per litre to 325 grams per litre of copper sulphate in acid solution (H_2SO_4, 50 g./l.), in nine steps of 25 grams. The amount of copper metal may be calculated from the factor given.

In order to enhance the difference between the varying depths of the same blue colour, a special screen is interposed in front of the solution. This has the effect of making the colours range from a purple to a blue grey, and in consequence small variations in colour are readily distinguishable.

A 5 mm. Lovibond Comparator cell must be used in conjunction with this disc.

Technique

The solution to be tested must be filtered bright before use, as a cloudiness due to suspended matter will render matching difficult.

Fill a 5 mm. cell with the solution, and place it in the right-hand compartment, so that it comes behind the screen and the clear centre of the disc. Hold the comparator facing a uniform source of white light—a north window whenever possible is best—and compare the colour of the solution with the colours in the standard disc, revolving the latter until a match is obtained. The value in grams per litre of copper sulphate is then read from the indicator recess. Intermediate values may be estimated.

To convert to ounces per gallon, divide the answer by $6\frac{1}{4}$. To convert to grams per litre of copper metal, divide the disc answer by 4 (or, more exactly, multiply by 0.254).

The Determination of Fluoride

using zirconium-alizarin

Introduction

A great deal of research has been carried out in America, and in this country, on the action of fluorine on dental caries. In many cases the indication has been that small quantities of fluorine, up to 1.0 p.p.m., are beneficial in the prevention of dental caries in young children. Excess, of course, is dangerous, and the question of fluoridation of public water supplies is still the subject of a certain amount of controversy.

Principle of the method

The determination of fluoride in water in quantities up to 1.5 p.p.m. is conveniently carried out by making use of the reaction between zirconium and alizarin, which produces a lake decolourized by fluorides. The method, developed by Sanchis and Scott[1] and Lamar[2], has been modified by Megregian and Maier[3], and a slight variation by Longwell[4] enables it to be employed for visual colour matching. The purity of both the alizarin and the zirconyl chloride is critical, particularly the purity of the alizarin, which must be free from sodium sulphate.

Reagents

1. Alizarin-zirconium reagent
   (a) Dissolve 0.420 g. of sodium alizarin sulphonate (Alizarin Red S) in 100 ml. distilled water.
   (b) Dissolve 0.25 g. zirconyl chloride ($ZrOCl_2.8H_2O$) in 100 ml. distilled water
   (c) Add 58.6 ml. sulphuric acid (analytical reagent grade) to 50 ml. of distilled water and cool.
   The mixed reagent is prepared by pouring (a) into (b) and adding (c), and diluting with distilled water to produce 1 litre.

2. Sodium arsenite solution
   Dissolve 1 g. of sodium arsenite in 100 ml. distilled water.

3. Sodium fluoride solution
   Dissolve 0.22 g. of sodium fluoride solution in distilled water and dilute to 1 litre. Dilute 10 ml. to 100 ml. with distilled water before use to give a solution of which 1 ml. = 0.01 mg. F.

It is essential to check the mixed alizarin-zirconium reagent with a standard fluoride solution and adjust it to match the disc before use. The prepared B.D.H. reagent (Code No. N1492) is already standardized in this way.

The Standard B.D.H. Lovibond Nessleriser Disc NOA

The disc (eight standards) covers the range from 0.4 to 1.1 parts per million of fluoride calculated as fluorine (F). A dulling screen is required.

Technique

Place 100 ml. of sample containing not more than 0.2 mg. fluoride in a 100 ml. Nessler cylinder and add 0.15 ml. of sodium arsenite solution (Reagent 2) to destroy any residual chlorine. Add 5 ml. of alizarin-zirconium reagent and allow to stand at 20° ± 2°C. for one hour. Place 50 ml. of the solution in a Nessleriser glass in the right-hand compartment of the Nessleriser, and 50 ml. of distilled water as a blank in the left-hand compartment. Stand the Nessleriser before a uniform source of white light — a north window is best — and compare the colour produced in the test solution with the colours in the standard disc, rotating the disc until a colour match is obtained.

Notes

1. Sulphates (SO_4) exceeding 200 p.p.m., orthophosphates exceeding 1 p.p.m., and metaphosphates at all levels give high results. Chlorides (Cl) exceeding 600 p.p.m., bicarbonate alkalinity ($CaCO_3$) exceeding 500 p.p.m., iron (Fe) exceeding 10 p.p.m., and aluminium at all levels give low results. Should any of the above radicals be present in larger quantities, it is necessary to carry out a Willard-Winter[5] steam distillation from perchloric acid to separate the fluoride[6].

2. B.D.H. Lovibond Nessleriser discs are standardized on a depth of liquid of 113 ± 3 mm. The height, measured internally, of the 50 ml. calibration mark on Nessleriser glasses used with the instrument must be within the same limits. Tests with Nessleriser glasses not conforming to this specification will give inaccurate results.

References

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3. S. Megregian and F. J. Maier, *J. Amer. W. W. Assn.*, 1952, **44**, 239
4. J. Longwell, *Proceedings of the Society for Water Treatment and Examination*, 1955, **4**, 44
5. H. H. Willard and O. B. Winter, *Ind. Eng. Chem. (Anal. Ed.)*, 1933, **5**, 7
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The Determination of Iron (1)

using thioglycollic acid

Introduction

The determination of iron is of interest in most laboratories. Iron is frequently found in natural water supplies and for most purposes it is considered that its concentration should be below 0.5 p.p.m. The presence of iron can be a considerable nuisance in water supplied for some industrial purposes, such as laundries and textile processing. The version of the thioglycollic acid test using the Nessleriser is sufficiently sensitive to enable control tests to be carried out at the required levels.

Principle of the method

The method adopted is that of matching the colour produced by the action of thioglycollic acid on iron, both ferric and ferrous, in the presence of ammonia. The most satisfactory results are obtained in the presence of alkali citrate, and the Lovibond colour glasses have been standardised on the colours produced in solutions containing ammonium citrate.

Reagents required

1. Solution of citric acid containing 20 per cent. w/v
2. Thioglycollic acid, Reagent quality
3. Solution of ammonia containing 10 per cent. w/w NH_3

The Standard Lovibond Comparator Discs 3/6 and APFE

Disc 3/6 covers the range 10γ to 100γ (0.01 mg. to 0.10 mg.) of iron (Fe), which equals 2.0 to 20.0 parts per million if a 5 ml. sample is taken.

Disc APFE covers the range 0.5-5.0 p.p.m. and must be used with a Special-Purposes Lovibond Comparator and 40 mm. cells.

Technique

To a suitable volume of the solution to be tested for iron, which should be neutral or slightly acid, add 0.5 ml. of a 20 per cent. w/v solution of citric acid; mix and add 1 drop of thioglycollic acid. Again mix, add 1 ml. of solution of ammonia, or sufficient to render distinctly alkaline and dilute with distilled water to 10 ml. Place this solution in one of the comparator test-tubes in the right-hand compartment of the comparator. In the left-hand compartment place the other test-tube containing a "blank" test composed of 0.5 ml. of the citric acid solution, 1 drop of thioglycollic acid, 1 ml. of solution of ammonia and sufficient distilled water to produce 10 ml. Hold the comparator facing a uniform source of white light — a north window wherever possible is the best — and compare the colour produced in the test solution with the colours in the standard disc, rotating the latter until a colour match is obtained. The amount of iron present in the test solution is then indicated in the right-hand corner of the comparator.

The markings on the disc represent the actual amounts of iron (Fe) producing the colour in the test; thus, if a colour equivalent to 0.05 mg. is produced when 5 ml. of the original solution is taken for the test, the amount of iron present in the original solution is 1 milligram per 100 ml. (0.001 per cent.) or 10 parts per million.

Alternative Technique

Using the greater optical depth afforded by the Nessleriser tubes it is possible to increase the sensitivity of the above test in the following manner: —

The Standard B.D.H. Lovibond Nessleriser Discs NEA, NEB and NEAB

Disc NEA covers the range 2γ to 18γ (0.002 to 0.018 mg.) of iron (Fe).
Disc NEB covers the range 20γ to 60γ (0.02 to 0.06 mg.) of iron (Fe).
Disc NEAB covers the range 5γ to 60γ (0.005 to 0.06 mg.) of iron (Fe).

Technique

Fill one of the Nessleriser glasses to the 50 ml. mark with distilled water; to this add 2 ml. of 20 per cent. solution of citric acid and 0.1 ml. of thioglycollic acid and render alkaline with ammonia (about 2 ml. of 10 per cent. ammonia solution). Mix and place in the left-hand compartment of the Nessleriser. Fill the other Nessleriser glass to the 50 ml. mark with the solution under examination, add 2 ml. of 20 per cent. solution of citric acid and 0.1 ml. of thioglycollic acid, mix and render alkaline with ammonia (about 2 ml. of 10 per cent. ammonia solution). After mixing, allow to stand for five minutes and then place in the right-hand compartment of the instrument. Stand the Nessleriser before a uniform source of white light — a north window is best — and compare the colour produced in the test solution with the colours in the standard disc, rotating the disc until a colour match is obtained.

The markings on the discs represent the actual amounts of iron (Fe) producing the colour in the test. Thus, if a colour equivalent to 10γ is produced in the test, the amount of iron present in the 50 ml. of solution under test is 0.01 mg. and the solution therefore contains 0.2 part of iron per million.

Notes

1. The reaction is not appreciably disturbed by relatively large amounts of lead, silver, tin, mercury, copper, cadmium, chromium, manganese, aluminium, calcium, magnesium, or the alkali metals, but any significant quantity of zinc depresses the intensity of the colour due to iron, while uranium, cobalt and nickel interfere. Sulphates, phosphates and nitrates do not disturb the test, but if sulphites are present it is necessary to apply a preliminary oxidation with potassium permanganate and to remove the excess of permanganate by treatment with oxalic acid before determining the iron. The test cannot be applied in the presence of cyanides.

2. B.D.H. Lovibond Nessleriser discs are standardized on a depth of liquid of 113 $\pm$ 3 mm. The height measured internally, of the 50 ml. calibration mark on Nessleriser glasses used with the instrument must be within the same limits. Tests with Nessleriser glasses not conforming to this specification will give inaccurate results.

The Determination of Iron (2)

using thiocyanate

Introduction

The thiocyanate method is more sensitive than that using thioglycollic acid and permits the determination of both ferrous and ferric iron if desired. Any ferrous iron present is oxidised to ferric by potassium permanganate, but the omission of this step enables the ferric iron to be determined separately. The ferrous iron can then be determined as the difference between ferric and total iron.

Principle of the method

The colour standards are designed to match the colours produced by the well-known reaction between ferric iron and ammonium thiocyanate in acid solution. In order to avoid the variation of colour which occurs in the presence of other metallic radicles, the coloured compound is extracted from aqueous solution by means of a mixed solvent consisting of equal volumes of amyl alcohol and amyl acetate.

Reagents required

1. Ammonium thiocyanate solution, approximately 7.5 M, containing 570 grams of ammonium thiocyanate (analytical reagent grade) per litre
2. Dilute hydrochloric acid, approximately 5 N
3. Potassium permanganate solution 0.1 N
4. A mixture of equal volumes of amyl acetate and amyl alcohol (both of analytical reagent grade)

The Standard Lovibond Comparator Disc 3/11

The disc covers the range from 2γ to 25γ (0.002 mg. to 0.025 mg.) of iron (Fe). This equals 0.4 to 5.0 parts per million if a 5 ml. sample is used.

Technique

Place 5 ml. of the solution under test into a small stoppered separating funnel, acidify with 1 ml. of dilute hydrochloric acid, add 2 drops of potassium permanganate solution and mix: then add 5 ml. of ammonium thiocyanate solution and 10 ml. of the mixed solvent. Shake vigorously and allow to separate. Reject the lower (aqueous) layer and transfer the upper layer to one of the 10 ml. comparator test tubes, and place in the right-hand compartment of the comparator. Carry out a blank test, using 5 ml. of distilled water with the quantities of the reagents stated above, and place the resulting solution in the left-hand compartment of the comparator, so that it comes behind the glass colour standards. Hold the comparator facing a uniform source of white light — a north window wherever possible is best — and compare the colour produced in the test solution with the colours in the standard disc, rotating the latter until a colour match is obtained. If the colour produced is deeper than the standard colours, the solution may be diluted with more of the organic mixed solvent until the colour is within the range of the disc, and the necessary calculation made. Alternatively, the test may be carried out on a smaller quantity of the original solution after dilution with distilled water to 5 ml.

The markings on the disc represent the actual amounts of iron (Fe) producing the colour in the test. Thus, if a colour equivalent of 20γ is produced when 5 ml. of the original solution is taken for the test, the amount of iron present in the original solution is 0.4 mg. per 100 ml. (0.0004 per cent.) or 4 parts per million.

Notes

1. Large quantities of phosphates or fluorides interfere with this reaction, but this interference may be avoided by increasing the quantity of dilute hydrochloric acid.

2. For details of a special kit for the estimation of iron as a part of the investigation of the mineral status of plants, apply to The Tintometer Ltd., Salisbury.

The Determination of Iron (3)

using *o*-phenanthroline

Introduction

The *o*-phenanthroline method for the determination of iron was originally proposed by Saywell and Cunningham[1] for application to fruit extracts and wines. Its use was extended by later workers[2], especially by Hammel and Willard[3] who applied it to the determination of iron in biological materials. The method has been extensively investigated by Fortune and Mellon[4] with special reference to factors influencing colour development and to the effect of potential interference from other ions.

The present method has been developed (Note 1) for the determination of iron in soil extracts. It is however generally applicable provided that due note is taken of the *p*H of the test solution and of the presence of possible interference from other ions (Note 5). The method is particularly useful for the determination of total and ferrous iron in natural waters.

Principle of the method

This determination is based on the orange-red complex $[(C_{12}H_8N_2)_3Fe]^{++}$ formed by *o*-phenanthroline with ferrous iron. Ferric iron is reduced to ferrous iron with hydroxylamine hydrochloride for the determination of total iron. The colour has been shown[4] to be independent of *p*H within the range 2–9. The intensity of this colour is proportional to the amount of iron present and is determined by comparison with Lovibond permanent glass standards.

Reagents required

1. ***Morgan reagent:***— Dissolve 100 g. of sodium acetate trihydrate (analytical reagent grade) in 800 ml. of distilled water, add 30 ml. of glacial acetic acid (analytical reagent grade) and make up to 1 litre.
2. ***Hydroxylamine hydrochloride:***— 10% aqueous solution (See Note 2)
3. ***Buffer solution:***— Mix equal volumes of 3*N* HCl and 5*N* ammonium acetate
4. ***o-phenanthroline:***— 0.5% solution in 50/50 alcohol/water mixture.

The Standard Lovibond Comparator Disc 3/54

This disc covers the range 10–140 micrograms of iron (Fe) and must be used with the dulling screen provided.

Technique

Take 10g. of soil in a 50ml. test tube, add 20ml. of Morgan reagent (reagent 1), shake for one minute, and filter through Whatman No. 1 filter paper. Transfer 5ml. of the filtrate to a standard comparator tube. Add 1ml. of distilled water, 1 ml. of hydroxylamine solution (reagent 2), mix well and allow to stand for 15 minutes. Then add 1ml. of buffer solution (reagent 3), 0.2 ml. of *o*-phenanthroline solution (reagent 4) and make up to the 10 ml. mark with distilled water. Mix well, using a thin glass rod bent at one end as a stirrer, and then place the tube in the right hand compartment of the Lovibond Comparator. Place an identical tube filled with distilled water in the left-hand compartment. After 20 minutes match the colour of the sample with the permanent glass standards in the disc, using north daylight wherever possible. The figure obtained represents micrograms of iron present in the volume of sample taken.

If the colour is darker than the highest standard, repeat the test using a smaller aliquot of the filtrate and increasing the initial addition of distilled water to maintain the volume of solution at 6 ml. before the addition of the hydroxylamine solution. Calculate the iron concentration proportionately.

Notes

1. This test was modified by A. G. Pollard, and W. Y. Magar, Imperial College, University of London.
2. Hydroxylamine hydrochloride solution is unstable and should be freshly prepared for each determination. It has been found that, instead of adding 1 ml. of a 10% solution, it is possible to add roughly 0.1 g. of the solid without any adverse effect on the determination. The same technique **cannot** be used with reagent 4, the *o*-phenanthroline **must** be used in the form of the specified solution, which is stable for at least 6 months if stored in a dark bottle with a ground glass stopper.
3. Except in the case of waterlogged soils rich in organic matter, there is no need to decolourise the soil extract.
4. It has been shown that up to 5 ml. of Morgan reagent has no effect on colour development. The colours are stable for up to 15 hours, and temperature, up to 30°C., has no appreciable effect on the results obtained.
5. The colour developed is independent of *p*H in the range 2–9. Silver and antimony give precipitates, cadmium mercury and zinc give slightly soluble complexes and reduce the intensity of the colour due to iron. The effect of these latter ions can be overcome by using excess reagent. If the sample is highly alkaline, add 2 drops of 10% acetic acid to the sample before developing the colour.

   The maximum amounts of interfering ions which may be present without causing error in the iron determination are :—

Cd	50 p.p.m.
Hg	1 (mercuric) 10 (mercurous) p.p.m.
Zn	10 p.p.m.
Be	50 p.p.m. if *p*H 3–5.5
Mo	no interference if *p*H > 5.5
W	5 p.p.m.
Ni	2 p.p.m.
Co	10 p.p.m. if *p*H 3–5
Sn	20 (stannous) p.p.m. if *p*H 2–3
	50 (stannic) p.p.m. if *p*H 2–5

6. This method estimates total iron. Ferrous iron may be determined, by omitting the reduction with hydroxylamine, and ferric iron may then be obtained by difference.

References

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4. W. B. Fortune and M. G. Mellon, *Ind. Eng. Chem. Anal. Edit.*, 1938, **10,** 60

The Determination of Lead (1)

using dithizone

Introduction

This method has been developed for the estimation of lead in urine and fæces, as an aid in assessing the degree of exposure to lead in workers in certain industries who have not developed evidence of plumbism.

The average excretion of lead in urine is between .003 and .005 mg. per 100 ml., while up to .01 mg. per 100 ml. may be considered to be within normal limits. In faeces an average of .25 mg. per 24 hours has been stated. Workers with lead may excrete as much as 10 times these amounts, while in cases of acute lead poisoning even these amounts are greatly exceeded.

Principle of the method

The lead is precipitated by entrainment with calcium oxalate. Interfering substances remain in solution in the supernatant fluid, which is discarded. The precipitate is digested with perchloric acid and the solution thus obtained is treated with ammonia and sodium cyanide. When the mixture is extracted with a chloroform solution of dithizone, a red colour is obtained which is proportional to the amount of lead present.

Reagents required, all analytical reagent grade

1. Dilute acetic acid Glacial acetic acid 10 ml. made up to 100 ml. with water
2. Nitric acid, fuming, sp. gr. 1.5
3. Ammonia solution .880
4. Dilute ammonia solution, 10 ml. of .880 ammonia made up to 100 ml. with water
5. Ammonium oxalate, saturated solution
6. Calcium chloride solution, 2.5 g. in 100 ml. water
7. Perchloric acid 60%
8. Citric acid solution, 10 g. in 100 ml. water
9. Dithizone solution, 4 mg. dithizone (diphenyl thiocarbazone) in 100 ml. chloroform This must be freshly made
10. Sodium cyanide solution, 5 g. in 100 ml. water
11. Chloroform
12. Indicators—Bromo-thymol blue and Bromo-cresol green

The Standard Lovibond Comparator Disc 5/17

The disc includes standards for the range 2γ to 20γ (0.002 to 0.02 mg.) of Lead (Pb), which will cover subjects exposed to lead hazards, and patients actually suffering from lead poisoning.

Technique for Lead in Urine

Adjust 10 ml. of the urine to approximately *p*H 4.5 by the addition of dilute acetic acid or dilute ammonia solution, using bromo-cresol green as indicator. Place in a centrifuge tube, add 1 ml. saturated ammonium oxalate solution and 0.5 ml. calcium chloride solution, stand for 20 minutes and then centrifuge. Decant the supernatant fluid, allow the tube to drain on a filter paper for 2—3 minutes, and digest the precipitate with 0.5 ml. perchloric acid until the solution is colourless. To the cooled contents of the tube add 1 ml. of water, 1 ml. dilute citric acid, 0.1 ml. of bromo-thymol blue indicator, and concentrated ammonia drop by drop until the mixture is just blue. Transfer to a 25 ml. separating funnel containing 1 ml. of the dithizone solution and 1 ml. of sodium cyanide solution, and shake until the red colour in the chloroform reaches a maximum. The chloroform layer

should then be run off and the extraction repeated with a further 1 ml. of dithizone solution. If the second chloroform extract shows an appreciable pink colour, a third or even a fourth extraction with 1 ml. portions of the dithizone solution must be made. The combined chloroform extracts are then placed in a separating funnel and shaken with 10 ml. of water, 0.1 ml. of sodium cyanide solution and 0.1 ml. of concentrated ammonia, when the green colour due to excess of reagent goes into the aqueous layer. Transfer the chloroform layer to a graduated vessel, make up to 5 ml. with chloroform, and place in a test-tube or 13.5 mm. cell in the right-hand compartment of the comparator. Parallel with the above work, a " blank " test is carried out, using 10 ml. of distilled water in place of urine : this blank is placed in the left-hand compartment of the comparator.

Hold the comparator facing a uniform source of white light—a north light when possible is best—and rotate the disc until the colour of the solution is matched by one of the Lovibond permanent glass standards. The value, expressed as mg. of lead (Pb) in the original volume of sample taken, is read from the indicator at the bottom right-hand corner. If the colour is deeper than the darkest standard, the test must be repeated using a smaller quantity of the original sample.

Technique for Lead in Faeces

Place 1 gram of dried faeces in a 500 ml. beaker. Carefully cover with pure fuming nitric acid and gently warm until the vigorous reaction has ceased. Evaporate the solution, with the addition of more nitric acid if necessary, until it consists of a dark brown clear liquid containing a little insoluble matter and a little fat. After cooling, the solution is diluted to 100 ml. and filtered, and 10 ml. of this solution are treated as above described for urine. The reading obtained from the comparator disc, multiplied by 10, will represent the amount of lead in the 1 gram sample taken, if the above quantities have been adhered to throughout. Appropriate adjustments must be made in the calculations according to any alterations made in the amount taken.

Note

All glassware used for this test must be scrupulously clean ; it should be boiled with nitric acid and thoroughly rinsed with distilled water before use.

References

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2. C. C. Lucas and J. R. Ross, *J. Biol. Chem.*, 1935, **111,** 285
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The Determination of Lead (2)

using sodium sulphide

Introduction

The toxic effects of lead are cumulative and the desirability of reducing lead contamination of foods to an absolute minimum is now widely recognised. Statutory limits range from 0.2 p.p.m. for " ready-to-drink " beverages to 20 p.p.m. for food colouring matters. The majority of foods are required to conform to a limit of 2 p.p.m.

Lead contamination of water, beer and cider has occasionally arisen as the result of the use of lead pipes and lead-glazed tanks : the dust of industrial atmospheres is often heavily contaminated with lead ; it has been found in tea packed in lead-lined chests and traces may remain from the use of certain insecticides. The following test was developed for the estimation of lead in food-colouring materials but may be used in other connections.

Principle of the method

This method is based on the colours produced by the addition of sodium sulphide to ammoniacal solutions containing lead. The conditions adopted for the standardisation of the colours are based upon the recommendations in the Second Report to the Analytical Methods Committee of the Society of Public Analysts and Other Analytical Chemists of the Sub-Committee on the Determination of Arsenic, Lead and other Poisonous Metals in Food-colouring Materials[1]. The presence of many other metals interferes with the sulphide test for lead and a high concentration of dissolved substances tends to influence the shade of colour produced, consequently it is usually necessary to isolate the lead before applying the colorimetric test. A method for the preliminary treatment of dyestuffs, which is applicable to organic material generally, is described in the Report referred to above. A more expeditious procedure using diphenyl-thiocarbazone has been described by Allport and Skrimshire[2] whereby it is possible to separate lead from organic substances and from all other common metals except bismuth and thallium. Samples which are colourless, such as water supplies, and do not contain a high proportion of dissolved solids, may sometimes be examined directly for lead provided they are free from other heavy metals. Owing to the use of potasium cyanide in the test solution, traces of copper and faint traces of iron do not interfere.

Reagents required, (all of analytical reagent grade)

1. A 10% w/v aqueous solution of ammonium acetate.
2. A 10% w/v aqueous solution of ammonium citrate prepared by dissolving 8.75 grams of citric acid in water, neutralising with ammonia and diluting with water to 100 ml.
3. A solution of ammonia containing 10% w/w NH_3
4. A 10% w/v aqueous solution of potassium cyanide.
5. A 10% w/v aqueous solution of sodium sulphide.

The Standard B.D.H. Lovibond Nessleriser Disc NF

The disc covers the range from 10γ to 100γ (0.01 mg.) of lead (Pb).

Technique

To a suitable quantity of the solution under examination (which should be approximately neutral and not exceed 25 ml. in volume) contained in a Nessleriser glass, add 10 ml. of ammonium acetate solution, 5 ml. of ammonium citrate solution, 5 ml. of ammonia solution and 1 ml. of potassium cyanide solution and dilute to 50 ml. with distilled water ; mix and then add 2 drops of sodium sulphide solution. Again mix and place in the right-hand compartment of the Nessleriser. In another Nessleriser glass place 10 ml. of ammonium acetate solution, 5 ml. of ammonium citrate solution, 5 ml. of ammonia solution and 1 ml. of potassium cyanide solution and dilute to 50 ml. with distilled water ; mix and add 2 drops of sodium

sulphide solution. Again mix and place in the left-hand compartment of the instrument. Stand the Nessleriser before a uniform source of white light — a north window wherever possible is the best — and compare the colour produced in the test solution with the colours in the standard disc, rotating the disc until a colour match is obtained.

The markings on the disc represent the actual amounts of lead (Pb) producing the colour in the test. Thus, if a colour equivalent to 30γ is produced in the test the amount of lead present in the quantity of solution taken for the test is 0.03 mg. of Pb.

Note

It must be emphasized that the readings obtained by means of the B.D.H. Lovibond Nessleriser and disc are only accurate provided that Nessleriser glasses are used which conform to the specification employed when the discs were being calibrated, namely that the 50 ml. calibration mark shall fall at a height of 113 mm. plus or minus 3 mm. measured internally.

References

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The Determination of Magnesium

using Titan Yellow

Introduction

Magnesium is one of the elements essential to plant growth, and appears in all plant and animal tissues. Its estimation is therefore of importance in the control of plant nutrient solutions and in the diagnosis of mineral deficiencies in crops.

Principle of the method

Of the numerous methods which have been advocated for the determination of traces of magnesium, that using Titan Yellow has given the most satisfactory results in horticultural work, and it does not suffer from interference by other radicles usually present in such solutions.

Titan Yellow is a very sensitive reagent for magnesium, and, in the concentration used in the test, shows a complete colour change with solutions containing from 0—10 parts per million of magnesium. The solutions to be tested must be diluted, therefore, with distilled water until the magnesium content lies within this range. Usually, in plant nutrient solutions, a ten or twenty-fold dilution will be satisfactory ; that is, 1.0 ml. diluted with distilled water to produce a volume of 10 or 20 ml.

Reagents required

1. ***Titan Yellow reagent*** This is prepared **freshly** from Titan Yellow solution (0·1 per cent in 20 per cent alcohol) as supplied by The British Drug Houses Ltd., Poole, by diluting with distilled water and standardising as described below. The stock solution should be kept in the dark. The diluted reagent deteriorates rapidly, and should always be freshly prepared for use.

2. Hydroxylamine hydrochloride (analytical reagent grade) .. .. 0.08 g.
   Sucrose (analytical reagent grade) .. .. 5.0 g.
   Distilled water to make .. .. 100 ml.

3. ***8N Sodium hydroxide solution.*** (32 g. NaOH in 100 ml.). This should be kept in small bottles, well closed with rubber bungs to prevent absorption of carbon dioxide from the atmosphere. It deteriorates on keeping, and should not be used when more than one month old, unless checked by titrating against a standard acid.

Preparation of Titan Yellow Reagent:

Slide the screen marked " Std. Titan Yellow " into the recess in the centre boss of the comparator. (An arrow will be seen on the screen moulding to indicate the direction in which the screen should be inserted). Fit the disc in the comparator and rotate it until " Std. Titan Yellow " appears in the indicator window of the comparator. The neutral grey standard in the disc now appears in front of the left hand cell compartment. Place a test tube of distilled water in the left hand compartment. In the right-hand compartment place a test tube containing 0·5 ml. (accurately measured) of B.D.H. Titan Yellow solution. Add distilled water from a graduated burette, until, after thorough mixing, the colour of the solution, viewed through the violet glass of the screen, matches the colour of the grey glass. Note carefully the volume of water used, to which mentally add 0·5 ml. (i.e. the volume of Titan Yellow solution used). This gives the final total volume required of the Titan Yellow reagent. Now prepare the reagent by diluting 1·0 ml. of the strong solution with sufficient distilled water to produce a volume equal to the final volume noted above. The reagent will thus be double strength to the solution prepared against the screen.

The Standard Lovibond Comparator Disc 3/28 (formerly APMG)

The disc contains colour standards for 0, 1, 2, 3, 4, 5, 7 and 10 parts per million of magnesium and one grey standard labelled " Std. Titan Yellow " for use with the screen marked

" Std. Titan Yellow " in standardising the Titan Yellow solution. There is also a second dulling screen, to be used when actually matching the unknown solution.

13·5 mm. cells or test tubes are used for this test.

Technique

In a clean test tube or 13·5 mm. cell place :

suitably diluted nutrient solution	3·0 ml.
hydroxylamine hydrochloride solution	0·5 ml. and mix well
Add Titan Yellow reagent, diluted as explained above ..	0·5 ml.
and then 2N Sodium hydroxide solution	0·5 ml.

Mix, and allow to stand for exactly five minutes from the last addition, then match in the comparator.

While waiting, place the screen marked " Magnesium " in the centre boss instead of the Titan Yellow screen. In the left-hand compartment place a test tube of distilled water, and in the right-hand one the test solution plus reagents.

Compare the colour of the test solution with the colours on the disc, rotating the latter until a colour match is obtained. If the colour is near to that marked 10 on the disc, the test should be repeated, using a more dilute nutrient solution, as there is little change in colour for concentrations above 10 p.p.m. of magnesium. The markings on the disc indicate parts of magnesium per million in the diluted solution ; therefore the result indicated must be multiplied by the dilution figure of the original nutrient solution.

Notes

1. Avoid using a mouth pipette for the sodium hydroxide solution. One with a rubber bulb is preferable. Where a reagent bottle with a pipette incorporated in the stopper is used for the sodium hydroxide, the solution as it gets low in the bottle leaves a white deposit of carbonate in the pipette where it is not immersed. This will detract for the accuracy of the pipette, and should be removed by washing with a 1 in 10 dilution of concentrated hydrochloric acid, followed by a thorough washing with distilled water.

2. The colours on the disc have been calibrated on the assumption that calcium is present in concentrations of not more than 500 p.p.m. Iron, manganese, aluminium, silica and proteins interfere if present in high concentrations. Phosphate in excess of 100 p.p.m. reduces the colour intensity somewhat.

3. For details of a special kit, incorporating this test, for the assessment of mineral deficiencies in plants, apply to The Tintometer Ltd., Salisbury.

References

1. G. S. Fawcett and R. H. Stoughton, "*The Chemical Testing of Plant Nutrient Solutions*", Tintometer Ltd., Salisbury, England, 1944

2. W. Plant, J. O. Jones and D. J. D. Nicholas, "*The Diagnosis of Mineral Deficencies in Crops by Means of Chemical Tissue Tests*", Annual Report of the Long Ashton Research Station, Bristol University, 1944

The Determination of Manganese (1)

using formaldoxime hydrochloride

Introduction

Manganese is essential to plant and animal life. Large amounts of manganese may however be toxic, and poisoning of plants grown on manganese-rich soils has been reported. The estimation of manganese is, therefore, of importance in agricultural chemistry.

Principle of the method

The procedure consists in the treatment of the manganese solution with formaldoxime hydrochloride, followed by the addition of 4N sodium hydroxide, until a wine-red coloration appears. The interference of iron is obviated by its extraction with ether after rendering the solution 6N with respect to hydrochloric acid.

Reagents required

1. A 5 per cent. aqueous solution of formaldoxime hydrochloride
2. 4N sodium hydroxide
3. Concentrated hydrochloric acid
4. Ether (s.g. 0.720)

The Standard Lovibond Comparator Disc 3/21

The disc covers the range from 5γ to 60γ (0.005 to 0.06 mg.) of manganese (Mn.).

Technique

To an aliquot part of the solution to be tested add 5 ml. of concentrated hydrochloric acid, and dilute to 10 ml. with water. Place in a separator, add 5 ml. of ether and shake. Transfer the aqueous layer to another separator, add another 5 ml. of ether, and shake again. Transfer the aqueous layer to a small (50 ml.) flask, and warm on a steam-bath to remove the dissolved solvent. Dilute the cooled solution to 50 ml. with water. To 10 ml. of this solution add 1 ml. of formaldoxime hydrochloride reagent, and sufficient 4N sodium hydroxide to produce the colour. Dilute to 15 ml. with water, mix, and transfer to a 13.5 mm. cell or test tube and place in the right-hand compartment of the comparator. In the left-hand compartment, place a similar cell of distilled water. With the comparator facing a north window rotate the disc until a match is obtained. The figure shown indicates the amount of manganese present in the solution actually taken for the test, and should be corrected by multiplying by the appropriate factor in order to obtain the value for the original solution.

Notes

1. Traces of copper produce a blue-violet colour with the reagent, but interference from this metal is obviated by adding a few drops of a 10% solution of potassium cyanide. The test is not influenced by the presence of relatively large concentrations of mercury, lead, bismuth, silver, cadmium, tin, antimony, arsenic, aluminium, zinc, or the alkaline earths. Molybdate, tungstate, and vanadate do not interfere; if beryllium is present the solution under test should be rendered alkaline with ammonium carbonate. Chromium, cobalt, and nickel produce colorations with the reagent, and in the presence of these metals the test for manganese is not applicable.
2. The formaldoxime method is not applicable to solutions which give precipitates when they are made alkaline. When this occurs the modified procedure suggested by Siderus[5] should be followed.
3. For details of a special kit, incorporating this test, for the assessment of the mineral deficiencies in plants, apply to The Tintometer Ltd., Salisbury.

References

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3. G. Deniges, *Compt. rend.*, 1932, **194**, 895
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The Determination of Manganese (2)

using sodium bismuthate

Introduction

This test, although not as sensitive as the formaldoxime test, has the advantage that it is specific for manganese. It is, therefore, of use with solutions containing chromium, cobalt, or nickel, which interfere with the formaldoxime method.

Principle of the method

This test is based on the permanganate colour produced by the oxidation of manganese by sodium bismuthate in nitric acid solution. To provide a blank solution for comparison when an inherently coloured solution is examined, the colour due to permanganate is discharged in a quantity of the test solution by hydrogen peroxide.

Reagents required, (all of analytical reagent grade)

1. Sodium bismuthate
2. Nitric acid
3. Hydrogen peroxide (20 volumes)

The Standard B.D.H. Lovibond Nessleriser Disc NG

The disc covers the range from 10γ to 90γ (0.01 to 0.09 mg.) of manganese (Mn.), and is used in conjunction with a dulling screen.

Technique

Boil 1 g. (or a suitable quantity) of the substance under test, which should be free from chloride, with 2 g. of sodium bismuthate, 15 ml. of nitric acid and 35 ml. of water until the reaction is **just** complete and a clear solution results. Cool rapidly under the tap, dilute with distilled water to 100 ml. and transfer to two 50 ml. Nessleriser glasses. Place one of these in the right-hand compartment of the Nessleriser; to the contents of the other add 2 drops of hydrogen peroxide, mix and place it in the left-hand compartment of the Nessleriser. Stand the Nessleriser before a uniform source of white light — a north window is best — and compare the colour produced in the test solution with the colours in the standard disc, rotating the disc until a colour match is obtained.

The markings on the disc represent the amount of manganese (Mn) producing the colour in the test. Thus, if a colour equivalent to 50γ is produced in the test carried out under the above conditions, using 1 g. of the substance being tested, the amount of manganese in 0.5 g. of the substance is 0.05 mg., which is equivalent to 100 parts per million.

Note

B.D.H. Lovibond Nessleriser discs are standardized on a depth of liquid of 113$\pm$3 mm. The height, measured internally, of the 50 ml. calibration mark on Nessleriser glasses used with the instrument must be within the same limits. Tests with Nessleriser glasses not conforming to this specification will give inaccurate results.

The Determination of Manganese (3)

using leucomalachite green

Introduction

The previous two methods for the determination of manganese are not sufficiently sensitive for use when manganese is present in trace amounts. The present method[1] has been developed for use in these circumstances. Although it was developed primarily for use in the determination of traces of manganese in soils, it is applicable to a wide range of materials provided that an appropriate extraction method is used to get the manganese into solution. It is particularly useful at low manganese concentrations where the classic periodate method fails.

Principle of the method

Manganese ions are oxidised to permanganate by potassium periodate. The permanganate ions oxidise the leuco base to the brilliant green carbinol[2]. The intensity of the green colour, which is proportional to the manganese concentration, is measured by comparison with Lovibond permanent glass standards.

Reagents required

1. ***Morgan's reagent*** (for soil extraction only).
   Dissolve 100 g. of sodium acetate trihydrate in 500 ml. of water. Add 30 ml. of glacial acetic acid and make up volume to 1 litre with water.

2. ***Buffer solution.***
   Dissolve 50 g. of sodium acetate trihydrate in 300 ml. of water. Add 400 ml. glacial acetic acid and make up to 1 litre with water.

3. ***Potassium periodate.*** 0.2% solution in water.

4. ***Leuco malachite green solution.***
   Dissolve 0.1 g. of leuco malachite green in 100 ml. of water containing 1.25 ml. of concentrated hydrochloric acid. Heat to 80°C. to help solution, taking care not to allow the temperature to exceed 80°. Cool and make up to 250 ml. with water.

All chemicals used in the preparation of these reagents should be of analytical reagent quality and distilled water should be used where water is required.

The Standard Lovibond Comparator Disc 3/55

This disc covers the range 0.03 to 0.3μgm. of manganese (Mn).

Technique

(a) ***Soil extraction.***
Shake 10 g. of soil with 20 ml. of Morgan's reagent (reagent 1) for 1 minute. Filter through Whatman No. 1 filter paper.

(b) ***Determination of manganese.***
Pipette 0.1 ml. of the sample solution, or the Morgan's soil extract, into a 10 ml. comparator tube, add 6 ml. of distilled water followed successively by 1 ml. of buffer solution (reagent 2) and 1 ml. of potassium periodate solution (reagent 3). Mix thoroughly, after each addition, by stirring the solution with a stirrer made from 4 mm. glass rod. Stand for 20 minutes, add 0.5 ml. of leuco malachite green solution (reagent 4) and dilute to the 10 ml. mark with distilled water. Mix thoroughly by stirring and place the tube in the right-hand compartment of the Comparator. Place an identical tube filled with distilled water in the left-hand compartment and match the colour of the test solution with the Lovibond permanent glass standards in the disc, using north daylight wherever possible. The figures obtained from the disc represent micrograms of manganese in the 10 ml. of solution in the tube, i.e. in 0.1 ml. of the sample solution. If the colour of the test solution is darker than that of the highest standard in the disc, then dilute the original solution, repeat the test, and multiply the final result by the dilution factor.

Notes

1. Owing to the extreme sensitivity of this test it is essential that great care be taken to prevent contamination of the glass-ware used with traces of manganese. All glassware should therefore be rinsed in standard chromic-sulphuric acid cleaning mixture immediately prior to the test and then thoroughly rinsed in distilled water.

2. The test should be carried out, if possible, at a temperature between 20°C and 30°C. At temperatures below 20°C colour development is slow. At temperatures above 30°C colour development is rapid and erratic. If ambient temperatures are above 30°C the comparator tube and its contents should be cooled during the period of colour development.

3. The colour is stable for two hours. After this time the colour gradually fades.

References

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The Determination of Nickel (1)

using ammonium citrate

Introduction

This test has been developed for the determination of nickel in plating solutions. In order to enhance the difference between varying depths of the same blue colour, a special screen is interposed in front of the test-tube containing the mixed nickel solution and reagent. This has the effect of making the colours range from pink through purple and grey to blue, and consequently small variations in colour are readily distinguishable.

Principle of the method

In the presence of ammonium salts, ammonium hydroxide forms a blue complex with nickel ions.

Reagent required

Citric acid	10 g.
Ammonia solution 10% w/w	60 ml.
Distilled water to produce	100 ml.

The Standard Lovibond Comparator Disc 3/14

The glasses in the disc are numbered 1 to 9, each colour corresponding to the amounts of nickel shown in the table.

A dulling screen must be used with this disc.

Technique

Place the dulling screen in position over the right-hand field.

Measure carefully the specified quantity (see table below) of the plating solution into one of the test-tubes, add 5 ml. of the reagent, fill the test-tube to the 10 ml. mark with water, mix well and place in the right-hand compartment of the comparator. In the left-hand compartment place a test-tube full of water. Hold the comparator facing a uniform source of light—a north window where possible is the best—and compare the colour produced in the solution with the colours in the disc, rotating the latter until a colour match is obtained. If the colour produced in the solution is weaker than the No. 1 standard glass or stronger than the No. 9 standard glass the test should be repeated using a larger or smaller quantity of the plating solution (see table).

Note

By carrying out the test on different quantities of the plating solutions, the standard disc may be used to determine the strengths of nickel ammonium sulphate solutions (nickel double salt) or of the much stronger nickel sulphate solutions (nickel single salt) normally used in electroplating. The accompanying table shows the quantities of the plating solutions which should be taken for the test according to the type of solution in use. Thus, if the plating solution contains nickel ammonium sulphate and 4 ml. be taken for the test, the range of the disc extends from 4 ounces to 12 ounces per gallon. If 3 ml. be taken for the test, the range of the disc is from 5⅓ ounces to 16 ounces per gallon. For the much stronger nickel sulphate solutions, correspondingly smaller quantities must be taken. Thus, the disc will cover the range from 16 to 48 ounces per gallon of crystallised nickel sulphate when 0.7 ml. of the plating solution is taken for the test.

If it is desired to know the strength of the solution in terms of metal nickel, then the quantities in the third section of the table should be taken. By the use of this table all calculations are avoided, and the content of the solutions may be obtained directly from a single observation.

Table.

Amount of the Plating Solution taken for test		Colour Standards No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9
3 ml.	Nickel Ammonium Sulphate.	5⅓	6⅔	8	9⅓	10⅔	12	13⅓	14⅔	16
4 ml.		4	5	6	7	8	9	10	11	12
0.7 ml.	Nickel Sulphate	16	20	24	28	32	36	40	44	48
1.4 ml.		8	10	12	14	16	18	20	22	24
2.1 ml.		5⅓	6⅔	8	9⅓	10⅔	12	13⅓	14⅔	16
2.8 ml.		4	5	6	7	8	9	10	11	12
0.6 ml.	Metal Nickel.	4	5	6	7	8	9	10	11	12
1.2 ml.		2	2½	3	3½	4	4½	5	5½	6
1.8 ml.		1⅓	1⅔	2	2⅓	2⅔	3	3⅓	3⅔	4
2.4 ml.		1	1¼	1½	1¾	2	2¼	2½	2¾	3

The figures in the chart represent ounces per gallon. To convert to grams per litre multiply by 6¼.

The Determination of Nickel (2)

using dimethyl-glyoxime

Introduction

In addition to its use in electro-plating other metals, nickel is extensively used industrially. It also occurs naturally in trace amounts in most plant and animal tissues. The following method can be adapted to the estimation of traces of nickel in alloys, minerals, chrome plating baths, fats obtained by hydrogenation processes involving a nickel catalyst, and as an impurity in cobalt salts.

Principle of the method

Nickel is often estimated gravimetrically by precipitation of the nickelous complex formed with dimethyl glyoxime. In the presence of oxidising agents however this divalent complex is converted into a soluble red tetravalent complex and this modified procedure serves as a very sensitive method for the estimation of traces of nickel.

Interference from small amounts of iron is prevented by the use of ammonium citrate. Appreciable quantities of other metals, including cobalt, copper and manganese, interfere and special procedures are necessary in their presence.

Reagents required

1. Ammonium citrate solution 10% w/v
2. Bromine water (saturated)
3. Ammonium hydroxide 10% w/w NH_3
4. Dimethyl-glyoxime 1% solution in Industrial Methylated Spirits

The Standard Lovibond Comparator Disc 3/36

The colours in the disc correspond to 1, 2, 3, 4, 5, 6, 7, 8 and 10 p.p.m. of nickel (Ni) in 10 ml. of the solution under test.

Technique

Fats and other organic materials must first be ashed, and the silica removed from siliceous minerals by evaporating to dryness with a mixture of perchloric and hydrofluoric acids. In each case the residue is then dissolved in dilute hydrochloric acid. Alloys are dissolved by heating with the appropriate mixture of acids. The final solution should be rendered neutral with ammonia.

To 10 ml. of solution under test add in the following order:—

5 ml. ammonium citrate solution,
2 ml. bromine water,
2 ml. ammonia solution,
1 ml. dimethyl glyoxime solution.

Mix thoroughly after each addition. Transfer to a Lovibond test tube or 13.5 mm. comparator cell and place in the right-hand compartment of the Lovibond Comparator. In the left-hand compartment place a blank of the reagents which has been prepared in an exactly similar manner.

Hold the comparator facing a uniform source of white light, a north window wherever possible, and compare the colour of the solution against the colours in the standard disc, rotating the latter until the nearest match is obtained.

The figure read in the indicator window represents parts per million of nickel in 10 ml. of the solution.

Notes

1. Interference can be caused either by the precipitation of a metal as the hydroxide or by complex formation between the interfering ion and the reagent.

In the former case separation can be effected by double precipitation or in certain cases by a special technique such as electrolysis.

When complex formation takes place, advantage can be taken of the fact that nickelous dimethyl glyoxime is soluble in chloroform whilst complexes formed with the majority of other metals are insoluble. Copper dimethyl glyoxime is soluble in chloroform, but unlike the nickel complex this can be decomposed with dilute ammonia. The nickel can then be back-extracted from the chloroform by treatment with dilute hydrochloric acid. This procedure is suitable for the estimation of nickel in cobalt salts.

2. It should be noted that in the presence of oxidising agents (e.g. manganese) a trace of hydroxylamine sulphate may be necessary to prevent the formation of the tetravalent nickel complex which is **insoluble** in chloroform.

References

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The Determination of Nitrate (1)

using phenol-2:4-disulphonic acid

Introduction

The estimation of nitrate is of importance in agricultural chemistry for the testing of soils; in the food industry for the control of pickling solutions and in the examination of meat products; and in the examination of both boiler-feed and potable waters. The following method is suitable for the examination of water supplies or foods if chlorides are removed as described.

Principle of the method

The test is based on the colour produced by the nitration of phenol-2:4-disulphonic acid according to the method originally suggested by H. Sprengel[1].

Reagents required

1. Phenol-disulphonic acid solution, approximately 25% w/v in concentrated sulphuric acid, made as follows:—
   Phenol (analytical reagent grade) .. .. 25 g.
   Concentrated sulphuric acid (nitrogen free) .. .. 158 ml.
   Dissolve and add
   Fuming sulphuric acid, containing about 20% sulphur trioxide .. .. 67 ml.
   Heat the mixture on a boiling water-bath for two hours.
2. Ammonia solution (analytical reagent grade) containing 10% w/v NH_3

The Standard Lovibond Discs, Comparator Disc 3/17 and Nessleriser Disc NHP

Disc 3/17 covers the range from 5γ to 100γ (0.005 mg. to 0.1 mg.) of nitrate nitrogen (N). This equals 1.0 to 20.0 parts per million if a 5 ml. sample is taken.

Disc NHP covers the range 2.5γ to 30γ (0.0025 to 0.03 mg.) of nitrate nitrogen (N).

Technique

The preliminary procedure will vary according to the character of the original material. Chlorides (see note at end), most forms of organic matter, and high concentrations of dissolved salts, must be absent.

Place a suitable quantity of the solution under examination in a small porcelain dish and evaporate to dryness on a boiling water-bath.

To the cooled residue add 1 ml. of the phenol-disulphonic acid solution, taking care that the reagent makes contact with the whole of the solid material derived from the sample, and allow to stand for 10 minutes. Then add 10 ml. of water, cool the mixture, add 10 ml. of ammonia solution, again cool, and dilute with water to 25 ml. At the same time, prepare a " blank " solution containing the same quantities of the reagents, omitting the sample under test. Fill one of the comparator test tubes with the test solution and place in the right-hand compartment of the comparator. Fill the other test tube with the " blank " solution and place in the left-hand compartment of the comparator.

Hold the comparator facing a uniform source of white light—a north window when possible is best—and compare the colour produced in the test solution with the colours in the standard disc, rotating the latter until a match is obtained. The matching of the colours should be carried out at 20°—25° C. The markings on the disc represent the nitrate nitrogen (N) in the amount of sample taken for the test (not the amount in the Comparator test tube). Thus, if a colour equivalent to 30γ is produced in a test carried out on 5 ml. of a sample of water, then the amount of nitrogen present as nitrate is 0.03 mg., which corresponds to 0.6 part per 100,000 of water.

For use with the B.D.H. Lovibond Nessleriser the procedure is identical except that the final volume is made up to 50 ml. and the measurements are carried out in standard Nessleriser tubes.

Notes

1. Nitrites in excess of 1 part per million will interfere.

2. In examining water supplies it will generally be necessary to remove chlorides, since interference from this source may be expected if the chloride content exceeds 0.2 part per 100,000. Chlorides may be removed by adding 1 ml. of glacial acetic acid to 10 ml. of the sample, followed by 0.1 gram of solid silver sulphate (nitrate free) and, after shaking, filtering through a Whatman No. 32 paper. The test for nitrate may then be applied to 5 ml. or other suitable quantity of the filtrate. The acetic acid is employed in order to prevent the adsorption of nitrate ions by the silver chloride precipitate.

Alternatively, a greater tolerance to the presence of chlorides is obtainable by a modification of this technique, described by Eastoe and Pollard,[2] see Nitrate (4) in this book.

3. For details of a special kit, incorporating this test, for the assessment of mineral deficiencies in plants, apply to The Tintometer Ltd., Salisbury.

References

1. H. Sprengel, *Pogg. Annalen*, 1864, **121,** 188
2. J. E. Eastoe and A. G. Pollard, *J. Sci. Food & Agric.*, 1950, 266

The Determination of Nitrate (2)

using 2:4-xylen-1-ol

Introduction

The following method is applicable in those instances where the presence of chlorides or nitrites interferes with the phenol-disulphonic acid method. It is particularly suitable for the examination of water and meat products.

Principle of the method

The test was originally suggested by Blom and Treschow[1] and depends upon the formation of 5-nitro-2:4-xylen-1-ol which is volatile in steam and can therefore be distilled into dilute sodium hydroxide with which it forms a highly coloured salt.

Reagents required

1. Alumina cream (see Note 2)
2. Lead acetate (basic) solution about 25% w/w
3. Sodium hydroxide solution 2N
4. Sulphuric acid 85% w/w (nitrogen free)
5. 2:4-Xylen-1-ol solution 1% w/v in glacial acetic acid
6. Silver sulphate (nitrogen free, analytical reagent grade)

The Standard B.D.H. Lovibond Nessleriser Disc NH

The disc covers the range from 2γ to 25γ (0.002 to 0.025 mg.) of nitrate nitrogen (N).

Technique

The preliminary procedure will vary according to the character of the original material. For some substances it will be sufficient to prepare an aqueous solution, or suspension, dilute to a known volume and take an aliquot part for the test. For the determination of nitrates present in water supplies, add 2 ml. of the sample to 4 ml. of distilled water and 15 ml. of 85% w/w sulphuric acid. Adjust the temperature of the liquid to 35° C. add 1 ml. of the xylenol solution and maintain the mixture at 35° C. for half an hour; then dilute with 100 ml. of distilled water and transfer to an ordinary distillation apparatus. Distil the mixture and collect 40 ml. in a Nessleriser glass containing 10 ml. of 2N sodium hydroxide. Adjust the temperature of the distillate to 20° C., and place the Nessleriser glass in the right-hand compartment of the Nessleriser. Fill another glass to the 50 ml. mark with water and place in the left-hand compartment of the instrument. Stand the Nessleriser before a uniform source of white light — a north window is the best — and compare the colour produced in the test solution with the colours in the standard disc, rotating the disc until a match is obtained.

The markings on the disc represent the actual amounts of nitrate nitrogen (N) producing the colour in the test. Thus, if a colour equivalent to 8γ is produced, the amount of nitrogen present as nitrate in the quantity of solution taken for the test is 0.008 mg.

The method is suited to the determination of nitrate present in meat products. For this purpose it is necessary to carry out the following procedure, which is a modification of that devised by McVey[2].

Transfer 1 g. of the minced sample to a 100 ml. volumetric flask containing 20 ml. of water, immerse the flask in a boiling water bath for 15 minutes and periodically agitate the contents. Allow the mixture to cool, render just acid with dilute sulphuric acid using bromocresol green as indicator, and oxidize any nitrites which may be present by adding 0.2 N potassium permanganate drop by drop until a faint pink colour persists for 1 minute. Add successively, with shaking after each addition, sufficient saturated aqueous solution of silver sulphate (analytical reagent grade) to precipitate any chloride present, 5 ml. of 25% w/v solution

of basic lead acetate and 5 ml. of alumina cream. Dilute with water to 100 ml. again shake and filter. Mix an appropriate proportion of the filtrate not exceeding 20 ml. (usually from 1 ml. to 5 ml. will be suitable) with three times its volume of 85% w/w sulphuric acid, adjust the temperature of the mixture to 35°C. add 1 ml. of the xylenol solution, maintain at 35°C. for half an hour, then dilute with 100 ml. of distilled water and transfer to an ordinary distillation apparatus. Distil the mixture and collect 50 ml. in a receiver containing 10 ml. of 2N sodium hydroxide and dilute the distillate to an appropriate volume. Transfer 50 ml. to a Nessleriser glass, adjust the temperature of the liquid to 20°C. and match the colour by means of the Nessleriser in the manner already described.

Notes

1. It is important that the temperature of distillates should be adjusted to 20° C., which is the temperature adopted for the standardisation of the nitrate disc, since the colour intensity of the nitro-compound in alkaline solution increases by 0.62% per 1° C.

2. The alumina cream is prepared by adding a slight excess of strong ammonia solution to a saturated aqueous solution of aluminium potassium sulphate (analytical reagent grade) and then cautiously adding more alum solution until the mixture is just acid to litmus.

References

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The Determination of Nitrate (3)

using brucine

Introduction

This method is in wide use for the estimation of nitrogen present as nitrate in boiler and river water.

Principle of the method

A solution of brucine, in concentrated suphuric acid, is nitrated by nitrates to give a deep red colour, fading to reddish-yellow.

Reagents required

1. Brucine 5 g. dissolved in 90 ml. glacial acetic acid and 10 ml. water
2. Sulphuric acid, concentrated, nitrogen free

The Standard Lovibond Comparator Disc 3/32

The disc covers the range 1 to 9 parts per million nitrogen (N) as nitrate. A dulling screen must be used with this disc.

Technique

To 1 ml. of sample add 0.1 ml. brucine solution and 2 ml. of sulphuric acid slowly from a dropping pipette. Shake, holding well away from the face and taking care not to spill any of the liquid on the hands, and allow to stand for 7 minutes.

Place in a Lovibond 13.5 m.m. cell or test tube in the right-hand compartment of the comparator, and use a blank of 1 ml. distilled water, plus the reagents in the above proportions, in a similar cell in the left-hand compartment. Hold the comparator facing a good north light and match against the colour standards in the disc. The dulling screen must be in place so that it comes in front of the test solution. Place a wedge of cotton wool in the right-hand compartment, so that the test-tube is raised and the small amount of solution covers the window.

The readings show parts per million N in the 1 ml. sample taken.

Notes

1. Organic substances in the quantities found in natural waters are not likely to interfere.
2. Chlorides in greater quantity than 1000 parts per million interfere, and must be removed by silver sulphate.
3. Nitrite interferes in proportion to its molecular weight, and should therefore be estimated separately and due allowance made. In most industrial waters the nitrite ion is present as less than 1 p.p.m., and in this concentration may be ignored.
4. Ammonia below 65 p.p.m. does not interfere.

Reference

L. W. Haase, *Chem. Ztg.*, 1926, **50**, 372

The Determination of Nitrate (4)

using phenoldisulphonic acid

Introduction

The nitrate ion plays an important role in plant nutrition and, for this reason, it is necessary to determine its concentration in soil samples. For this purpose, the procedure developed in 1911 by Chamot and Pratt[1–4], using phenoldisulphonic acid, has been used as the standard method by many workers. This 'standard' procedure has, however, many disadvantages including that of the time required, interference by chloride ion, and the precipitation of some metallic hydroxides and phosphates under the test conditions.

The present test[5] has been developed to overcome these difficulties. In addition details are given of a rapid procedure which, while being slightly less accurate and also rather more susceptible to interference by chloride ions, nevertheless is a useful method when rapid assessments of the nitrate concentrations of soils are required in the field, where some of the facilities required for the more accurate test would not be available.

Principle of the method

Phenol-2:4 disulphonic acid is nitrated by nitrates to 6-nitrophenol-2:4-disulphonic acid, which gives rise to an intense yellow colour on the addition of alkali. The intensity of this colour, which is proportional to the nitrate concentration, is determined by comparison with Lovibond permanent glass standards.

Reagents required

1. 20% *(w/w) phenoldisulphonic acid*

Phenol (analytical reagent grade)	25 g.
Sulphuric acid (analytical reagent grade)	100 ml.
Fuming sulphuric acid, 20% (w/w) SO_3	67 ml.

Dissolve the phenol in the sulphuric, slowly stir in the fuming sulphuric acid, and heat the mixture in a water bath at 100°C. for 2 hours (Note 1).

2. *Diluted phenoldisulphonic acid*

Slowly add 100 ml. of reagent 1 to 40 ml. of distilled water. Keep the mixture cool and well stirred by swirling the flask under running water. This diluted reagent is supersaturated with respect to solid phenoldisulphonic acid at ordinary temperatures. Should crystals separate, they may be redissolved readily by placing the bottle containing the reagent in warm water. For this reason it is preferable to dilute reagent 1 as required.

3. *Diluting solution*

Ammonium citrate (pure)	5 g.
0.880 ammonia solution	125 ml.
Distilled water	to 1 litre

The Standard Lovibond Comparator Disc 3/56

The disc covers the range 10–200 μg. of nitrate nitrogen.

Technique

(a) ***Laboratory method*** Pipette 1 ml. of the soil extract into the bottom of an ordinary thin-walled test-tube, and add 1.5 ml. of reagent 2 down the side of the tube from a small burette. Thoroughly mix the contents of the tube, by gently twirling the tube between the fingers, and then place the tube in a vigorously boiling water bath. Take care that the part of the tube containing the reaction mixture is immersed in the water.

After 3 minutes remove the tube from the water bath, and dilute almost to the 25 ml. mark with diluting solution (reagent 3). Mix well, cool to 20°C. in a water bath, dilute to the mark, mix again and transfer to a 13.5 mm. comparator tube or rectangular comparator cell. Place in the right-hand compartment of the comparator and match against the permanent Lovibond glass standards in the disc, using north daylight wherever possible.

(b) ***Rapid method*** To 1 ml. of the soil extract in a dry test-tube, graduated at 25 ml., add 1 ml. reagent, running the reagent straight into the extract. Shake gently, set the tube aside for 2 minutes, dilute to the mark with reagent 3 and match against the disc as in (a) above.

Notes

1. 20% (w/w) phenoldisulphonic acid reagent (reagent 1) may be obtained ready made if preferred. Details may be obtained from Tintometer Ltd.
2. It has been shown[5] that varying the time of heating or the temperature of the final solution both affect the intensity of the colour produced. The details of the technique given must therefore be followed exactly.
3. This modification of Chamot and Pratt's original method does not differentiate between nitrate and nitrite, both ions producing the same colour. If it is required to estimate nitrate in the presence of nitrite, then the original method[1] should be used.
4. The following are the maximum amounts in micrograms of ions which can be tolerated without causing interference in the determination of nitrate.

Br^-	2	Na^+	6,000	Fe^{2+}	15
I^-	10	K^+	4,000	SO_3^{2-}	80
NH_4^+	2,000	Fe^{3+}	200	Cu^{2+}	500

References

1. E. M. Chamot and D. S. Pratt, *J. Am. Chem. Soc.*, 1909, **31,** 922
2. *idem. ibid.*, 1910, **32,** 630
3. *idem. ibid.*, 1911, **33,** 366
4. *idem. ibid.*, 1911, **33,** 381
5. J. E. Eastoe and A. G. Pollard, *J. Sci. Food and Agric.*, 1950, No. 9, 266

The Determination of Nitrate (5)

using Nessler's reagent

Introduction

This test[1] was developed to provide a simple and reliable method for the determination of nitrate in water, with special reference to the needs of the brewing industry. The test is, however, appropriate to any solution containing nitrate and can be applied to any material provided that an effective method of extracting the nitrate is used. As the final stage of the determination uses the standard Nessleriser discs for determining ammonia, this enables both nitrate and ammonia determinations to be carried out with the same equipment and on the same sample if required.

Principle of the method

Nitrate ions are reduced to ammonium ions by boiling with chromous chloride solution. Ammonia is released from the ammonium ions, by making the solution alkaline, and removed by steam distillation. The distillate is reacted with Nessler's reagent producing a yellow colour, the intensity of which is proportional to the ammonia concentration, and is measured by comparison with Lovibond permanent glass standards.

This method does not differentiate between nitrate and nitrite ions. If significant concentrations of nitrite ions are present in the samples, the nitrite concentration must be determined separately[2] and allowance made for it in the calculation of the nitrate concentration.

Reagents required

All chemicals used should be of analytical reagent quality. Water used in preparing reagents should be distilled or deionised, and should be free of ammonia.

1. ***Chromous chloride solution.*** This reagent is prepared[3] by the reduction of potassium chromate using nitrogen-free zinc in the following manner :— Wash granulated zinc by boiling for a few minutes with hydrochloric acid diluted with an equal volume of water. (This operation must be carried out in an efficient fume cupboard). Rinse thoroughly with water. Place 2g. of potassium dichromate, 10 ml. of concentrated hydrochloric acid, 5 ml. of water and a large excess (about 10g.) of the purified zinc, in a 125 ml. conical flask. Boil for 1 to 2 minutes until the colour of the solution becomes pale blue. Cool immediately, and cover the surface of the liquid with a layer of petroleum ether (b.p. 100—120° C.) to inhibit oxidation. This reagent must be prepared immediately before use.

2. ***Caustic soda solution.*** Dissolve 200g. of sodium hydroxide in water and dilute to 1 litre in a 2 litre Pyrex flask. Heat to boiling, add an excess of chromous chloride solution (10 to 20 ml.), boil the solution for about 30 minutes, filter through a Whatman No. 54 filter paper, cool and adjust the final volume to 1 litre.

3. ***Nessler's reagent.*** (See Note 1). Dissolve 35g. of potassium iodide and 12.5g. of mercuric chloride in 800 ml. of water. Slowly add a cold saturated solution of mercuric chloride until, after repeated shaking, a slight red precipitate remains. Add 120g. of sodium hydroxide ; shake until dissolved and finally add a little more of the saturated solution of mercuric chloride. Make up to 1 litre with water. Allow to stand, with occassional shaking for several days. Use the clear supernatant liquid for the tests.

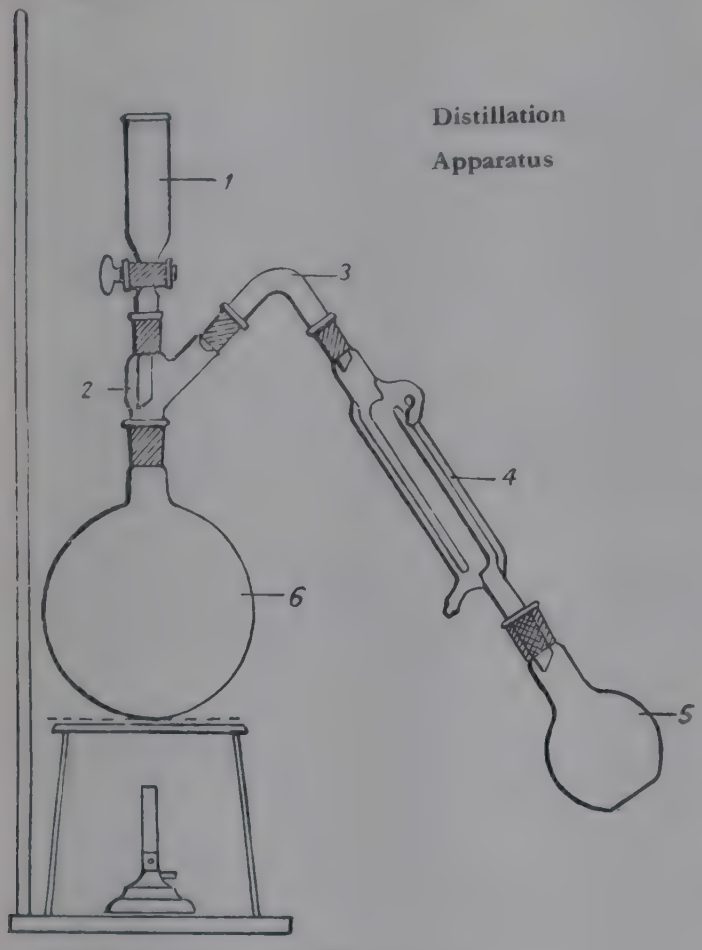
The Standard Lovibond Nessleriser Discs, NAA, NAB, NAC and NAD

Disc NAA covers the range 1 μg. to 10 μg. of ammonia (NH_3).
Disc NAB covers the range 10 μg. to 26 μg. of ammonia (NH_3).
Disc NAC covers the range 28 μg. to 60 μg. of ammonia (NH_3).
Disc NAD covers the range 60 μg. to 100 μg. of ammonia (NH_3).

The conversion of ammonia concentration to the equivalent nitrate concentration is discussed in Note 4.

Technique

Pipette 50 ml. of the water sample into a 1 litre flask, and add 200 ml. of ammonia-free distilled water. Connect the flask to a distillation apparatus fitted with a dropping-funnel (See figure).



Distillation Apparatus

1. Dropping funnel Cat. No. D. 3/12
2. Multiple adaptor Cat. No. M.A. 2/3
3. Bend Cat. No. S.H. 1/22
4. Double surface condenser. Cat. No. C. 5/12
5. 500 ml. Pyrex, flat bottom flask, medium neck B. 24
6. 1,000 ml. Pyrex, round bottom flask, short neck B.24

Also required :— B. 24 stopper B. 19 stopper

Catalogue numbers refer to :—
Quickfit & Quartz Ltd., Mill Street, Stone, Staffs.

Turn off the water supply to the condenser and heat the solution until steam emerges from the end of the condenser. Turn on the condenser cooling water and collect the distillate. Continue heating until 50 ml. of distillate have been collected. Test this preliminary distillate for ammonia, in the manner described below for the final distillate, and then discard it. If the preliminary distillate gives a positive reaction with the Nessler reagent, collect a further 50 ml. and test this.

When the preliminary distillate has been shown to be free of ammonia pipette 1 ml. of chromous chloride solution (reagent 1) into the flask and boil for about 15 seconds to ensure thorough mixing. Add 2 ml. of caustic soda solution (reagent 2) and continue heating until two successive 50 ml. portions have been collected in Nessler tubes (see Note 2). To each tube add 2 ml. of Nessler's reagent (reagent 3 and Note 1) and mix. Place one of the tubes in the right-hand compartment of the Nessleriser. Prepare a blank on the reagents by carrying out the whole of the above procedure but omitting the 50 ml. of sample and substituting an additional 50 ml. of distilled water, i.e. 250 ml. in all. By using replicate distillation equipment the blank and samples can be treated simultaneously. For the blank collect only the first 50 ml. of the final distillate, add Nessler's reagent as above, and place in the left-hand compartment of the Nessleriser. Compare the colour of the sample with the permanent Lovibond glass standards in the disc, using north daylight wherever possible. Note the measurement and repeat with the second fraction of the sample distillate. Add these two disc readings together.

This combined figure gives the total content of reducible nitrogen (nitrate plus nitrite) in the sample, expressed as micrograms of ammonia. This can be converted to micrograms of nitrate (NO_3^-) by multiplying by 3.65 or by use of the Table (see Note 5).

The presence of nitrite in the sample may be detected by any suitable qualitative test, of which the Tofranil test[3] is probably the simplest. If nitrite is shown to be present, its concentration can be determined by the standard Nessleriser Nitrite test[2] and due allowance made for its contribution to the final answer.

The concentration of ammonia in the original sample can be obtained by adding 2 ml. of Nessler's reagent directly to a fresh 50 ml. of the sample in a Nessler tube and measuring, using the same discs[2]. In this case the disc reading should be quoted directly and not referred to the conversion Table. Neither is there any need to correct the final answer for the initial ammonia content as the 'free' ammonia is removed by distillation before the production of ammonia by reduction of nitrates and nitrites.

Notes

1. The published formulae for Nessler's reagent vary considerably. When using the Lovibond permanent glass standards for the determination of ammonia it is important that the reagent employed should correspond to that used for preparing the original standard colours. The formula used for that purpose is given above (reagent 3) and this must be followed exactly.

2. Readings obtained by the use of the B.D.H.-Lovibond Nessleriser and discs are accurate only provided that the Nessler tubes used conform to the specification employed when the discs were calibrated, namely that the 50 ml. calibration mark shall fall at a height of 113 $\pm$ 3 mm. when measured internally.

3. If it is found that the colour which develops in the first 50 ml. of the final distillate falls outside the range of the discs, then the test should be repeated, using either less or more sample as appropriate, and making up to 250 ml. total volume with distilled water as before. The final result is then related to the volume of sample used. If the result is divided by the sample volume then the figure obtained will be in micrograms per ml., i.e. in parts per million (w/v).

4. A possible source of interference is albuminoid matter in the water, but the conditions of this test have not proved sufficiently severe to cause decomposition of the albumin to ammonia in any of the waters yet examined.

5. **Conversion Table**

Disc	*Reading* (μg. NH_3)	*Equivalent nitrate concentration* (μg. NO_3^-)
NAA	1	4
	2	7
	3	11
	4	15
	5	18
	6	22
	7	26
	8	29
	10	36
NAB	12	44
	14	51
	16	58
	18	66
	20	73
	22	80
	24	87
	26	95
NAC	28	102
	32	117
	36	131
	40	146
	44	160
	48	175
	52	190
	56	204
NAD	60	219
	65	237
	70	255
	75	273
	80	292
	85	310
	90	328
	95	346
	100	365

References

1. G. A. F. Harrison, *Talanta*, 1962, **9**, 533.
2. "*Colorimetric Chemical Analytical Methods*". The Tintometer Ltd, Salisbury, England.
3. A. Irudayasamy and A. R. Natarajan, *Analyst*, 1962, **87**, 831.

The Determination of Nitrite

using Griess-Ilosvay's reagent

Introduction

Nitrites frequently appear with nitrates in the pickles and brines used for preserving meat. Nitrates are not regarded as preservatives in the relevant regulations,[1] but when they are used for pickling meat, nitrites are formed by bacterial reduction of the nitrate. The permitted content of nitrite in cooked pickled meat is limited to 200 p.p.m., calculated as sodium nitrite.[1] The estimation of nitrites is also of importance in the analysis of water, as the presence of nitrite may indicate contamination of the water by sewage.

The following test is designed for the determination of nitrites in water, and can be adapted for the estimation of nitrites in other materials.

Principle of the method

The well known Griess test,[2] as modified by Ilosvay,[3] depends on the diazotisation of sulphanilic acid by nitrous acid. The compound thus formed is then coupled with 1-naphthylamine to produce a red azo dye.

Reagents required (all of analytical reagent grade)

Various formulæ for the reagent have been suggested and experiment has shown that the colours produced by these vary slightly; it is therefore important to employ a reagent corresponding with that used for standardising the colours. The following formula, which is that quoted in *The Examination of Water and Water Supplies*, (Thresh-Beale-Suckling, 7th edition, 1958, by E. Windle Taylor), has been adopted.

(1)	Sulphanilic acid	0.5 g.
	Acetic acid glacial	30 ml.
	Water redistilled	120 ml.

Dissolve and filter.

(2)	1-Naphthylamine	0.1 g.
	Acetic acid glacial	30 ml.
	Water redistilled	120 ml.

Dissolve the 1-naphthylamine in the acetic acid, add the water and filter.

Should either of the above solutions become coloured on keeping, shake with a little zinc dust and refilter.

The Standard B.D.H. Lovibond Nessleriser Disc NJ

The disc covers the range 0.05γ to 1γ (0.00005 to 0.001 mg.) of nitrogen (N) present as nitrite.

Technique

Fill one of the Nessleriser glasses to the 50 ml. mark with distilled water and place in the left-hand compartment of the Nessleriser. Fill the other Nessleriser glass to the mark with the water under examination, add 2 ml. of Griess-Ilosvay's solution No. 1 and an equal quantity of solution No. 2, mix, allow to stand for 15 minutes, and then place in the right-hand compartment of the instrument. Stand the Nessleriser before a uniform source of white light — a north window is the best — and compare the colour produced in the test solution with the colours in the standard disc, rotating the disc until a colour match is obtained. Should the colour in the test solution be deeper than the standard colour glasses, a fresh test should

be carried out using a smaller quantity of the water under examination and diluting to 50 ml. with distilled water before adding the reagent. The sample when tested must be at a temperature of 20° C.

The markings on the disc represent the actual amounts of nitrogen (N) present as nitrite producing the colours in the test. Thus, if on adding the reagent to 50 ml. of water, a colour equivalent to 1γ is produced, the amount of nitrogen present as nitrite in the water will be 0.02 part per million.

Note

It must be emphasized that readings obtained by the B.D.H. Lovibond Nessleriser and discs are only accurate provided that Nessleriser glasses are used which conform to the specification employed when the discs were calibrated, namely that the 50 ml. calibration mark shall fall at a height of 113 mm. plus or minus 3 mm. measured internally.

References

1. The Public Health (Preservatives etc., in Food) Regulations 1925—40
2. J. P. Griess, *Ber.*, 1879, **12**, 426
3. L. Ilosvay de Nagy Ilosua, *Bull. Soc. Chim.*, 1889, **49**, 388

The Determination of Nitrite (2)

using Griess-Ilosvay's reagent

Introduction

This is a modification of method (1) which has been adapted for the determination of nitrite in meat pickling brines, and reads direct in percentages of sodium nitrite.

Principle of the method

Sulphanilic acid is diazotised with nitrous acid and the resulting compound is coupled with 1-naphthylamine, producing a red azo dye.

Reagents required: all chemicals to be analytical reagent grade

1.	Dialysed iron	about 5% Fe_2O_3
2.	Sodium chloride	10% in distilled water
3.	Sulphanilic acid reagent (Griess Ilosvay's reagent No. 1)	
	Sulphanilic acid	0.5 g.
	Acetic acid, glacial	30.0 ml.
	Distilled water	to 120 ml.
4.	1-naphthylamine reagent (Griess Ilosvay's reagent No. 2)	
	1-naphthylamine	0.18 g.
	Acetic acid, glacial	30 ml.
	Distilled water	to 120 ml.

Dissolve the 1-naphthylamine in the acetic acid, add the water, and filter. Should either of the reagents 3 or 4 become coloured during storage, shake with a little zinc dust and filter.

The Standard Nessleriser Disc NOJ

The disc covers the range 0.01 to 0.05% sodium nitrite ($NaNo_2$) in 9 steps of 0.005%. A dulling screen must be used with this disc.

Technique

Pipette 5 ml. of well-mixed brine into a 500 ml. volumetric flask, add about 300 ml. of distilled water, 2.5 ml. of dialysed iron, make up to the mark with distilled water, mix well, and filter. Pipette 5 ml. of the filtrate into each of two 100 ml. volumetric flasks, to each add 5 ml. of Reagent 2, and 50 ml. of distilled water. Mix well. To each flask add 2.0 ml. of Reagent 3, and to one only add 2.0 ml. of Reagent 4. Make contents of both up to 100 ml. with distilled water, mix, and allow to stand for 45 minutes. Transfer 50 ml. of each solution to Nessleriser tubes, and place in the right-hand compartment of the Nessleriser, the tube containing Reagents 3 and 4. Place in the left-hand compartment the "blank" from which Reagent 4 was omitted. Compare the colours against disc NOJ using a uniform source of white light, either North daylight or the special White Light Cabinet made for use with the Nessleriser.

Note

It must be emphasised that the readings obtained by means of the B.D.H. Lovibond Nessleriser and disc are only accurate provided that Nessleriser cylinders are used which conform to the specification employed when the discs were calibrated, namely, that the 50 ml. calibration mark shall fall at a height of 113 mm. $\pm$ 3 mm. measured internally.

References

1. The Public Health (Preservatives etc., in Food) Regulations 1925—40
2. J. P. Griess, *Ber.*, 1879, **12**, 426
3. L. Ilosvay de Nagy Ilosua, *Bull. Soc. Chim.*, 1889, **49**, 388

The Determination of Noxious Nitrogen

using copper acetate

Introduction

This method has been developed for the estimation of noxious nitrogen in the sugar beet.

The term " noxious nitrogen " refers to the nitrogen in those substances not eliminated during the normal purification processes in the beet sugar factory. These nitrogenous constituents can have a pronounced influence on the ultimate extraction efficiency, and the measure of their prevalence in the beet itself must therefore be of considerable value. Of the total harmful nitrogen, that present in the form of amino acids is regarded as the most objectionable.

Principle of the method

This method, due to Stanek and Pavlas, makes use of the blue colour produced by amino acids in the presence of copper acetate.

Reagent required (all of analytical reagent grade)

Dissolve 10 g. of copper nitrate in 700 ml. distilled water, and add 250 g. sodium acetate (cryst.). Prepare solution in the cold, then make up to 1,000 ml. and filter.

The Standard Lovibond Comparator Disc 3/27

The disc covers the range 10—90 mg. Nitrogen in 100 g. Sugar Beet and is designed for use with the Special Purposes Comparator and 1″ cell. Owing to the brightness of the solutions, a special dulling screen must be used with this disc.

Technique

To 2 ml. of the copper reagent add 20 ml. of freshly filtered clarified solution of half-normal strength, prepared by the customary digestion method from beet brei or cossettes, and which contains no added acetic acid. Place the well-shaken coloured solution in the 1″ cell in the right-hand compartment of the comparator, so that it comes behind the centre of the disc. Rotate the disc until the nearest colour match is obtained. The figure shown in the aperture at the lower right-hand side of the comparator face gives the value for the sample under test in milligrammes noxious nitrogen per 100 gms. beet, or parts per hundred thousand. Readings should be taken facing north daylight, or, failing this, with a suitable light source to reproduce the same conditions. As the filtrates tested are perfectly clear and colourless, no second cell is necessarily required in the left-hand side of the comparator, but for greater ease in reading, distilled water may be used as a blank.

Notes

1. The standard colours for calibrating this disc have been prepared by using a solution of mono-sodium glutamate of such strength that 1 ml. made up to 100 ml. with distilled water gives, on addition of 10 ml. of the special copper reagent, a blue colour equivalent to 10 mg. Nox. N per 100 g. beet.

2. This disc was prepared with the co-operation of the Central Laboratory of the British Sugar Corporation, Ltd., and their help is gratefully acknowledged.

Reference

Stanek & Pavlas: *Z. Zuckerind čechoslovak.*, Rep. **59**, 129 (1934/35); **60**, 46 (1935/36)

The Determination of Oxygen (1)

using indigo-carmine

Introduction

The indigo-carmine test was developed by Buchoff, Ingber and Brady[1] for the colorimetric determination of low concentrations of dissolved oxygen in water. This method has been modified for use with the B.D.H. Lovibond Nessleriser and Lovibond permanent glass standards by Alcock and Coates[2] using a special reaction vessel. The results of this modified test have been shown by the authors to be in good agreement with those obtained using a Hersch cell[3] and meter, and also with the *o*-tolidine test.[4] The method is exceptionally neat and quick, and should prove valuable for routine control work on boiler feed waters, particularly in the "field."

Principle of the method

Reduced indigo-carmine is a bright yellow-green colour. On oxidation it changes to orange, to red, to purple, to blue, and finally to blue-green in the completely oxidised form. The indigo-carmine is reduced by glucose in the presence of potassium hydroxide. Glycerol is used to provide air-stability and to sharpen the colours.

Reagents required

1. Indigo-carmine stock solution :

Indigo-carmine—reagent grade	0.018 g.
Glucose—reagent grade	0.20 g.
Distilled water	to 5 ml.
Glycerol—reagent grade	75 ml.

This reagent is stable for 90 days if stored in a refrigerator, and for 6 weeks at ambient temperature if stored in the dark.

2. Potassium hydroxide 37.5 g.
   Distilled water 62.5 g.

3. Leuco reagent
   In a small bottle mix 8 ml. of reagent 1 and 2 ml. of reagent 2. After thorough mixing allow the reagent to stand until the initial dark red colour changes to lemon yellow (approximately 10 minutes). The leuco reagent should be freshly prepared on the day it is to be used.

The Standard B.D.H. Lovibond Nessleriser Disc NOE

The disc covers the range 0 to 0.120 ml./l. oxygen (O_2). The disc must be used in conjunction with the special glass reaction vessel supplied. To convert ml./l. to parts per million, multiply the result by 1.43.

Technique

The sample must be taken and the reagent added in the absence of atmospheric oxygen. For this purpose a special glass reaction vessel has been developed for use with the B.D.H. Lovibond Nessleriser (see Figure). Sampling may be carried out by the " Submerged bottle Method "[5] which is probably the most reliable method to use. However, with experience, a satisfactory sample can be obtained by slowly lowering the reaction vessel from the end of a submerged and rigid vertical sampling tube through which the water is flowing at a rate of about a litre per minute at room temperature.

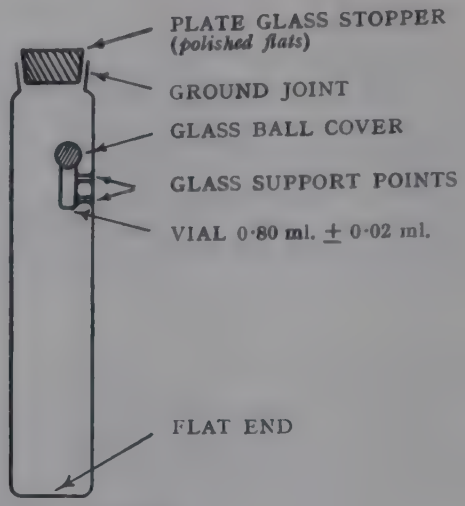
With a dropping pipette the vial attached to the inner surface of the special reaction vessel is filled with leuco reagent (reagent 3). Any entrapped air bubbles are allowed to rise, and disperse, so that the vial is completely full and free of air. It is then sealed by means of a glass

ball which may be placed in position by sliding the ball down a glass tube placed over the end of the vial. The sample is allowed to flow through the reaction vessel for at least 10 minutes, to remove any reagent on the outside of the vial. The reaction vessel is then removed from the sampling line and the stopper inserted, under water, to prevent the trapping of any air.

NESSLER
REACTION VESSEL

for use in the B.D.H.

Lovibond Nessleriser



60 *ml. capacity*

The reaction vessel is now removed for the water ; inverted to allow the ball to fall off the vial, and the contents thoroughly mixed by shaking. Five minutes after mixing, the reaction vessel is placed in the right-hand compartment of the B.D.H. Lovibond Nessleriser and a standard Nessleriser tube filled with the sample without reagents is placed in the left-hand compartment to serve as a blank. The colour developed in the sample is then compared with the colours of the permanent glass standards, using a good north light.

Notes

1. The preparation of the indigo-carmine reagent can be simplified by the use of special indigo-carmine-glucose tablets prepared by B.D.H. Ltd., Poole, Dorset.
2. No interference has been found from nickel, cupric or zinc ions in concentrations of 1 p.p.m. nor from ferric ion at 3 p.p.m. Sulphite and hydrazine do not interfere at the low concentrations normally found in boiler feed water. Of the metals tested, only ferrous ion has been found to interfere. This ion, or intolerable concentrations of other ions, can be removed by a mixed bed ion-exchange demineraliser in series with the sampling line.
3. The temperature of the sample must not exceed 70°F (21°C.).
4. The adaptation of this test to the Nessleriser is due to the "Alfloc" Water Treatment Service of I.C.I. Ltd., to whom acknowledgment is made.

References

1. L. S. Buchoff, N. M. Ingber and J. H. Brady, *Analyt. Chem.*, 1955, **27**, 1401
2. G. P. Alcock and K. B. Coates, *Chem. & Indust.*, 1958, 554
3. P. Harsch, *Nature, (Lond.)*, 1952, **169**, 792
4. B.S. 1427, 1949, *Tests for water used in steam generation*
5. B.S. 2690, 1956, *Methods of testing water used in industry*

The Determination of Dissolved Oxygen (2)

using Winkler's method

Introduction

This is a method of estimating the amount of dissolved oxygen in river water in the field. The test is easily performed and gives sufficiently accurate results for most purposes.

It will be of interest to workers in many fields of research, including :—

River Survey Boards
Municipal Surveyors and Engineers
Fish Breeders
Fishery and Catchment Boards
Water Works Engineers
Biological Laboratories
Medical Officers of Health

Principle of the method

In the Winkler method[1, 2, 3] for the determination of dissolved oxygen in water, the sample contained in a completely filled bottle is treated with a solution of manganese chloride and a solution containing potassium hydroxide and potassium iodide. Manganese hydroxide is precipitated and this absorbs the dissolved oxygen present in the sample, forming the higher oxides of manganese. On subsequently acidifying the mixture with sulphuric acid, the higher oxides of manganese react with the potassium iodide present, liberating iodine in an amount equivalent to that of the dissolved oxygen originally present. Instead of titrating the liberated iodine as in the original Winkler method, the amount present may be determined by measuring the depth of the yellow colour produced. This can be carried out readily by comparing the colour with a series of Lovibond glasses standardised on the colours produced by known quantities of iodine liberated in the course of the determination of dissolved oxygen by the Winkler method.

Reagents required

Ampoules (a) white glass containing manganese chloride 0.2 g., distilled water (air free) to 2 ml.

(b) amber glass containing potassium iodide 0.3 g., potassium hydroxide 1.0 g., distilled water (air free) to 2 ml.

Concentrated sulphuric acid

Compressed tablets of potassium iodide, 5 grain

Standard Lovibond Discs, Comparator Disc 3/3, and Nessleriser Discs NKA and NKB

Disc 3/3 covers the range from 0.4 to 1.2 parts of dissolved oxygen per 100,000 by weight, which equals 2.7 to 8.3 ml. per litre.

Disc NKA : for the determination of dissolved oxygen in boiler feed water and covering the range from 0.05 to 1.0 ml. of dissolved oxygen per litre (0.007 to 0.14 part per 100,000).

Disc NKB : for the determination of dissolved oxygen in river water and other natural waters and covering the range from 0.04 to 0.16 part of dissolved oxygen per 100,000 ; by conducting the test as set out below, the range may be extended to 1.6 parts per 100,000.

In order to increase the range of utility of the disc the yellow solution obtained in the test, may, if necessary, be suitably diluted so as to bring the colour within the range of the standard glasses. Simple dilution with water, however, is not satisfactory, as this also reduces the concentration of the potassium iodide, and the yellow colour of the iodine-potassium iodide compound is destroyed and replaced by the much paler brown colour of iodine. It is therefore essential to add potassium iodide when diluting the solution. The potassium iodide can be added conveniently in the form of a compressed tablet.

Technique

A wide necked glass bottle which contains 200 ml. when full, having a ground-in glass stopper, is filled to the brim with the water to be examined. The temperature of the sample is noted. Make a file mark near each end of one of the white ampoules and one of the amber ampoules. Break off one tip of the white ampoule, place the open end of the ampoule under the surface of the water in the bottle and then break off the other tip, thus allowing the contents to flow into the bottle. Transfer the contents of the amber ampoule to the bottle in the same manner, insert the stopper in the bottle so that no bubble of air is trapped, invert the bottle several times in order to mix the contents thoroughly and allow to stand for five minutes for the precipitate to settle. Remove the stopper and by means of a pipette add rapidly 2 ml. of concentrated sulphuric acid, the tip of the pipette being just below the surface of the water : again stopper the bottle and mix the contents thoroughly by inverting the bottle several times.

Comparator

One 10 ml. glass tube is filled with an untreated sample of the water and the other tube with the treated solution under examination. The former is placed in the left-hand compartment of the comparator and the latter in the right-hand compartment.

The comparator is held about 18 inches from the eyes facing a source of white light ; north daylight is to be preferred and direct sunlight must be avoided. The bakelite disc is revolved until the two apertures show the same colour, and the amount of dissolved oxygen in parts per hundred thousand is read at the indicator recess near the right-hand bottom corner of the comparator.

Nessleriser

With the brown solution thus obtained fill one of the Nessleriser glasses to the 50 ml. mark and place it in the right-hand compartment of the Nessleriser. In the left-hand compartment place 50 ml. of the untreated water to which has been added a tablet of potassium iodide and 0.5 ml. of analytical reagent grade sulphuric acid. (In case the of a water which has been proved to remain unaltered in colour on the addition of potassium iodide and sulphuric acid, it will be sufficient if the Nessleriser glass in the left-hand compartment is filled with the untreated water). Stand the Nessleriser before a uniform source of white light—a north window wherever possible is the best — and compare the colour in the test solution with the colours in the standard disc, rotating the disc until a colour match is obtained.

Notes

1. Should the colour of the treated water be deeper than that of the standard glasses, an aliquot of the treated water should be diluted with the untreated water, and after a tablet of potassium iodide and 0.1 ml. of concentrated sulphuric acid have been added, the colour of the resulting solution should be compared with the standard glasses.

When it has been necessary to dilute the treated water before matching the colour, the figures indicated should be multiplied by the corresponding dilution figure.

2. Many natural waters contain iron, which in the ferric state will liberate iodine from an acidified solution of potassium iodide. To allow for any yellow colour due to iodine from this source, it is advisable to add a tablet of potassium iodide and 0.1 ml. of concentrated sulphuric acid to the untreated water under test and to use this solution in the tube in the left-hand compartment instead of an untreated blank.

3. By dividing the oxygen content, ascertained by this method, by the concentration of dissolved oxygen necessary to saturate the water at the temperature of sampling (these values can be obtained from published tables), and multiplying by 100, the percentage saturation is obtained.

4. If a more sensitive test is required, Weir's modification of the Winkler method, described on a separate page, should be used.

5. The preparation of the necessary disc was suggested by members of the staff of the Ministry of Agriculture and Fisheries Research Station at Alresford, who assisted in its standardization.

References

1. L. W. Winkler, *Ber.*, 1888, **21,** 2843
2. S. Rideal and W. T. Burgess, *Analyst*, 1909, **34,** 193
3. N. L. Allport, "*Colorimetric Analysis*", London, Chapman and Hall, 1945

The Determination of Dissolved Oxygen (3)

using Weir's method

Introduction

The importance of proper de-aeration in preventing corrosion in steam plants working at high temperatures and pressures is now universally accepted. In a paper entitled "Estimation of dissolved oxygen in de-aerated water," Arnott and McPheat described a method for estimating oxygen in quantities down to 0.001 ml. per litre. At the request of Messrs. G. & J. Weir, Ltd., Nessleriser discs have been prepared for use with this method.

Principle of the method

The method is essentially that due to Winkler[1], but it has been so modified[2] that the iodine liberated is measured colorimetrically and not titrated as in the original procedure.

Water contained in a completely filled bottle is treated with a solution of manganese chloride and a solution containing potassium hydroxide and potassium iodide. Manganese hydroxide is precipitated and this absorbs the dissolved oxygen present in the sample, forming the higher oxides of manganese. On subsequently acidifying the mixture with sulphuric acid, these react with the potassium iodide present, liberating iodine in a amount equivalent to that of the dissolved oxygen originally present. The iodine is extracted by shaking with carbon tetrachloride and the colour matched in the Nessleriser with a disc containing a series of yellow glasses, each of which has been carefully matched to correspond to a definite concentration of iodine. The oxygen content of the water is indicated by the figure on the disc corresponding to the standard colour which matches that of the test solution.

Boiler water often contains copper and ferric iron and, as these will liberate iodine from potassium iodide, it is essential to carry out a blank determination. In an acid solution, potassium iodide is unaffected by the presence of dissolved oxygen; thus, by adding the reagents in the reverse order, the iodine liberated is equivalent solely to the interfering ions.

Reagents required

White glass ampoules each containing 0.2 g. of manganous chloride in sufficient air-free water to produce 2 ml.

Amber glass ampoules each containing 0.3 g. of potassium iodide and 1 g. of potassium hydroxide in sufficient air-free water to produce 2 ml.

Sulphuric acid (analytical reagent grade)

Carbon tetrachloride (analytical reagent grade)

The Standard B.D.H. Lovibond Nessleriser Discs NYA and NYB

The following two Nessleriser discs are available :—

Disc NYA covers the range 0.001—0.020 ml. dissolved oxygen per litre (0.00014—0.0028 parts per 100,000).

Disc NYB covers the range 0.025—0.100 ml. dissolved oxygen per litre (0.0035—0.014 parts per 100,000). A dulling screen is required with these two discs.

Technique

The water used in the test should be cooled by an efficient coil to below 65°F. and preferably to a temperature less than that of the surrounding air. This ensures that the air is not subsequently drawn in by contraction of the sample and also facilitates the easy withdrawal of the stopper.

Fill a bottle, of about 1,110 ml. capacity and fitted with a ground-in glass stopper, with the water to be tested. In order to avoid contamination from atmospheric oxygen it is necessary to fill the bottle by means of a tube passing to the bottom and to allow the water to overflow so as to displace the air in the bottle and also the water which has come in contact

with the air ; the tube should be withdrawn whilst the water is still running. Make a file mark near each end of one of the white ampoules and one of the amber ampoules. Break off one tip of the white ampoule, place the open end of the ampoule under the surface of the water in the bottle and then break off the other tip, thus allowing the contents to flow into the bottle. Transfer the contents of the amber ampoule to the bottle in the same manner, insert the stopper in the bottle so that no bubble of air is trapped, invert the bottle several times in order to mix the contents thoroughly and allow to stand for five minutes. Remove the stopper and by means of a pipette add rapidly 2 ml. of sulphuric acid AnalaR ; again stopper the bottle and mix the contents thoroughly by inverting the bottle several times. Transfer 1,000 ml. to a separating funnel and extract by shaking twice with 20 ml. portions of carbon tetrachloride and, finally, with a further 10 ml.

Pour the combined extracts into a Nessleriser glass and place it in the right-hand compartment of the Nessleriser.

A blank is carried out by treating a further 1,100 ml. of the water under test in a similar manner except that the reagents are added in the **reverse** order. The combined extracts are placed in the left-hand compartment of the Nessleriser.

Stand the Nessleriser before a uniform source of white light — a north window is the best — and compare the colour in the test solution with the colours in the standard disc, rotating the disc until a colour match is obtained.

The figures on both Disc NYA and Disc NYB indicate millilitres of dissolved oxygen per litre.

Notes

1. It is essential that the water be completely free from suspended matter and also from sulphites. Prior to and during a de-aeration test, the sulphite supply should be cut off. When there is any doubt about the purity of the water it is advisable to verify that it does not absorb iodine. In order to test for absorption, a measured amount of iodine solution is added to a litre of acidified water, extracted with 50 ml. of carbon tetrachloride and matched on the disc. For example, if 8.00 ml. of N/1000 iodine were added initially, then the colour of the carbon tetrachloride extract should correspond to 0.045 ml. per litre of oxygen.

2. For the estimation of higher concentrations of dissolved oxygen in water, the original Winkler method is suitable.

3. It must be emphasised that readings obtained by the B.D.H. Lovibond Nessleriser and disc are only accurate provided that Nessleriser glasses are used which conform to the specification employed when the discs were calibrated, namely that the 50 ml. mark shall fall at a height of 113 mm. plus or minus 3 mm. measured internally.

References

1. L. W. Winkler, *Ber.*, 1888, **21,** 2843
2. J. Arnott and J. MacPheat, *Engineering*, 1953, **176,** 103

The Determination of Phosphate (1)

using Deniges' method

Introduction

Small amounts of phosphate have frequently to be determined in the presence of silicate, and under many conditions of test this silicate causes considerable interference. Colour standards have been prepared for use in conjunction with a modification of Deniges' method which is insensitive to the presence of silicates up to 700 parts per million in the solution under test. The method is probably the most sensitive known for the colorimetric determination of phosphate, and is relatively free from interference by other substances.

Principle of the method

The blue colour produced is obtained by the reduction of phosphomolybdic acid with freshly prepared stannous chloride solution. As the depth of colour is dependent on the proportion of added reagents, temperature, and time of reaction, it is essential that strict attention be paid to all details in order to obtain correct results.

Reagents required

1. Ammonium Molybdate (analytical reagent grade) .. .. 10 g.
   Distilled Water to produce .. .. 100 ml.
   When dissolved, add slowly to a cooled mixture of
   Sulphuric Acid (analytical reagent grade) .. .. 150 ml.
   Distilled Water .. .. 150 ml.

This reagent will keep indefinitely if stored in a resistance glass bottle and protected from light.

2. Pure Tin Foil .. .. 0.1 g.
   Hydrochloric Acid (analytical reagent grade) .. .. 2 ml.
   Copper Sulphate 4 per cent solution .. .. 1 drop

Warm in a test tube until no more tin dissolves, cool, and dilute to 10 ml. with distilled water. This stannous chloride reagent **must** be freshly prepared.

The Standard Lovibond Discs, Comparator Disc 3/7 and Nessleriser Disc NMB

Disc 3/7 covers the range from 20γ to 220γ (0.02 to 0.22 mg.) of phosphate calculated as P_2O_5. This equals 2.0 to 22.0 parts per million if a 10 ml. sample is used. For smaller amounts of phosphate, use the B.D.H. Lovibond Nessleriser and the disc employing the same method.

Nessleriser Disc NMB covers the range from 2γ to 20γ (0.002 to 0.02 mg.) of phosphate calculated as P_2O_5. This disc requires the use of a dulling screen.

The markings on the disc represent the actual amounts of phosphate, calculated as phosphoric oxide (P_2O_5), producing the colour in the test. Thus if a colour equivalent to 10γ is produced in the test, the amount of P_2O_5 present in the volume of solution taken for the test is 0.01 mg.

Technique

Dilute a suitable volume of the solution under test with distilled water to 45 ml.; transfer to a flask immersed in a water-bath at 25° C. When the solution has reached the temperature of the bath, add 1 ml. of the ammonium molybdate reagent and mix thoroughly. Then add 0.15 ml. of the stannous chloride reagent, mix, and dilute to 50 ml. with water at 25° C. and allow to stand for five minutes.

Comparator

Transfer 10 ml. to a test tube and place in the right-hand compartment in the comparator; fill a test tube with the untreated solution under test, diluted with distilled water in the same proportions, and place in the left-hand compartment, to provide a background for the glass colour standards. Hold the comparator facing a uniform source of white light — a north window wherever possible is best — and compare the colour produced in the test solution with the colours in the standard disc, rotating the latter until a colour match is obtained. If the colour produced is deeper than the standard colours, the test should be repeated, using a smaller volume of the solution under examination.

The markings on the disc represent the actual amount of phosphate, calculated as phosphoric oxide (P_2O_5) producing the colour in the test. Thus, if a colour equivalent to 20γ is produced, the amount of P_2O_5 present in the volume of solution taken for the test is 0.02 milligram.

Nessleriser

For use with B.D.H. Lovibond Nessleriser place the whole 50 ml. in a Nessleriser glass in the right-hand compartment of the Nessleriser and in the left-hand compartment place a Nessleriser glass containing the same volume of the solution under test diluted with distilled water to 50 ml. Stand the Nessleriser before a uniform source of white light — preferably a north window — and compare the colour produced with the colours in the standard disc, rotating the disc until a colour match is obtained. Should the colour in the test solution be deeper than the standard colour glasses, a fresh test should be carried out using a smaller quantity of the solution under examination.

The markings on the disc represent the actual amounts of phosphate, calculated as phosphoric oxide (P_2O_5), producing the colour in the test. Thus if a colour equivalent to 10γ is produced in the test, the amount of P_2O_5 present in the volume of solution taken for the test is 0.01 mg.

Notes

1. The colour is produced only in the presence of ortho-phosphates: meta and pyro-phosphates must therefore be completely hydrolysed before testing. Free mineral acids and alkalis depress the colour and must therefore be neutralised. Certain organic acids, such as citric, oxalic, and tartaric (but not acetic acid), inhibit the development of the blue colour if present in appreciable amounts. If a substantial amount of organic matter is present it must be removed, to avoid possibilities of interference. Ferric iron if exceeding 1 part per million in the final test solution, should be reduced to the ferrous state, for which purpose a Jones's reductor is recommended in the paper quoted. Arsenate must be reduced to arsenite, by means of hydrogen sulphide in acid solution.

2. It must be emphasised that readings obtained with the B.D.H. Lovibond Nessleriser and disc are only accurate provided that Nessleriser glasses are used which conform to the specification employed when the discs were calibrated, namely that the 50 ml. mark shall fall at a height of 113 ± 3 mm. measured internally.

Reference

W. M. Holman and A. G. Pollard, *J.S.C.I.*, 1937, **56**, 339T

The Determination of Phosphate (2)

using a modification of Tschopp's method

Introduction

The following method is a variant of the molybdate method, using metol instead of hydroquinone as reducing agent. Metol is claimed to be less sensitive to the presence of silicates, which give a similar reaction to phosphates, than any other reducing agent.

Principle of the method

The test is based on the colours produced by the reduction of phosphomolybdic acid with *p*-methylamino-phenol sulphate (metol). Numerous variations of this test have been proposed from time to time and many different reducing agents have been recommended. The technique here adopted is a modification of that advocated by Ernst and Emilio Tschopp, which experience has shown to be most reliable for use in conjunction with permanent standards since, under the conditions of the test, uniform colours are produced, and the test is rendered relatively insensitive as silica.

Reagents required

1. Ammonium molybdate (analytical reagent grade) .. .. 10 g.
   Distilled water to produce .. .. 100 ml.
   Dissolve and add slowly to a cooled mixture of
   Sulphuric acid (analytical reagent grade) .. .. 150 ml.
   Distilled water .. .. 100 ml.
   This reagent will keep indefinitely if stored in a resistance glass bottle and protected from light.
2. Sodium metabisulphite .. .. 40 g.
   Sodium sulphite (analytical reagent grade) .. .. 1 g.
   Metol .. .. 0.2 g.
   Redistilled water (analytical reagent grade) to produce .. .. 200 ml.

The Standard Lovibond Discs, Comparator Disc 3/4 and Nessleriser Disc NMC

Disc 3/4 covers the range from 5γ to 100γ (0.005 to 0.1 mg.) of phosphate calculated as phosphorus pentoxide (P_2O_5). This value multiplied by 0.4367 gives the value in terms of phosphorus (P).

Disc NMC covers the range 5γ to 60γ (0.005 to 0.06 mg.) of phosphate calculated as phosphorus pentoxide (P_2O_5).

Technique

(a) ***Comparator***

To 5 ml. of the solution under examination, contained in one of the graduated test-tubes, add 1 ml. of Reagent No. 1 and heat the tube in a boiling water-bath for 15 minutes. Then add 1 ml. of Reagent No. 2 and continue the heating in a boiling water-bath for a further 15 minutes. Cool the solution, dilute it with distilled water to 10 ml. and place it in the right-hand compartment of the comparator. At the same time and in the same manner prepare a blank solution from 5 ml. of distilled water and place in the left-hand compartment of the comparator. Hold the comparator facing a uniform source of white light — a north window wherever possible is best — and compare the colour produced in the test solution with the colours in the standard disc, rotating the latter until a colour match is obtained.

The markings on the disc represent the actual amounts of phosphate, calculated as phosphorus pentoxide (P_2O_5) producing the colour in the test. Thus, if a colour equivalent to 60γ is produced in the test carried out under the above conditions, the amount of P_2O_5) present in 5 ml. of the original solution is 0.06 mg. and the solution therefore contains 12 parts of P_2O_5 per per million.

(b) *Nessleriser*

To 25 ml. of the solution under examination, or a suitable quantity diluted to 25 ml., contained in one of the Nessleriser glasses, add 5 ml. of Reagent No. 1 and immerse the Nessleriser glass in a boiling water-bath for 15 minutes; then add 5 ml. of Reagent No. 2 and continue the heating for a further 15 minutes. Cool the solution, dilute it with distilled water to 50 ml. and place it in the right-hand compartment of the Nessleriser. At the same time and in the same manner prepare a blank solution from 25 ml. of distilled water and place it in the left-hand compartment of the Nessleriser. Stand the Nessleriser before a uniform source of white light — a north window is the best — and compare the colour produced in the test solution with the colours in the standard disc, rotating the disc until a colour match is obtained.

The markings on the disc represent the actual amounts of phosphate, calculated as phosphoric oxide (P_2O_5), producing the colour in the test. Thus, if a colour equivalent to 50γ is produced in the test carried out under the above conditions, the amount of P_2O_5 present in 25 ml. of the original solution is 0.05 mg. and the solution therefore contains 2 parts of P_2O_5 per million.

If the colour produced in the test is deeper than the standard colours, the original solution should be suitably diluted and the test carried out on the diluted solution, the result being corrected accordingly.

Notes

1. Nitrates may interfere, if present to the extent of more than 1 mg. NO_3, using the Comparator, or more than 3 mg. using the Nessleriser.

2. The test is five thousand times less sensitive to silica than to phosphate.

3. Iron can be tolerated in concentrations up to one thousand times the amount of phosphate.

4. It is essential that the solutions to be examined for phosphate should be neutral, since uniformity of colour production is dependent on the final concentration of free acid in the solution. If the solution contains a buffer salt such as sodium acetate, a quantity of sulphuric acid equivalent to the acetate must be added before carrying out the test.

5. Arsenites react with phosphates to give a similar colour and should be removed by precipitation with hydrogen sulphide followed by boiling and filtration. Sugars, and also lactates, citrates, tartrates, oxalates and other organic salts, depress the intensity of colour produced by phosphates, and these compounds if present should be removed.

6. It must be emphasised that the readings obtained with the B.D.H. Lovibond Nessleriser and disc are only accurate provided that Nessleriser glasses are used which conform to the specification employed when the discs were calibrated, namely that the 50 ml. mark shall fall at a height of 113 ± 3 mm. measured internally.

Reference

E. & E. Tschopp, *Helv. Chim. Acta.*, 1932, **15**, 793

The Determination of Phosphate (3)

using amino-naphthol-sulphonic acid

Introduction

This method has been developed for the estimation of phosphate in blood and urine, but the techniqne may be modified for the determination of phosphate in water used in steam generation (see Note at end).

Normal adult blood contains 2—3 mg. of inorganic phosphate* per 100 ml., while the blood of children contains somwehat more, about 4—5 mg. per 100 ml. In conditions of acidosis, such as nephritis, the blood phosphate is often raised. In children suffering from rickets there is a decrease of the inorganic phosphate : the amounts in plasma are almost the same as those in whole blood. The inorganic phosphate is usually determined in a trichloracetic acid extract of the blood or plasma.

An acid extract of blood contains, in addition to the inorganic phosphate, esters of phosphoric acid i.e. a form of combined phosphate. There is only a small amount, less than 1 mg. of ester phosphate per 100 ml., in the plasma. The cells contain 50—80 mg. The esters of phosphoric acid are hydrolysed by enzymes of the blood to liberate inorganic phosphate : thus, in blood which has been drawn for some time, the inorganic phosphate will be found to have increased. It is therefore necessary that freshly drawn blood be used for the determination of free and ester phosphate. The ester phosphate is low in rickets.[2, 4]

Principle of the method

The method is based on King's modification[3] of the Fisk and Subbarrow procedure.[1] The inorganic phosphate (i.e. the phosphoric ion in the trichloracetic extract of blood) is combined with molybdic acid, and reduced with amino-naphthol-sulphonic acid to give a blue colour, which is proportional to the amount of phosphate ion. The ester phosphate is determined in the same way after destroying the organic matter and liberating free phosphate, perchloric acid being used for this purpose.

The amount of blue colour estimated in the solution after heating with perchloric acid gives the measure of the total phosphate i.e. the inorganic plus the ester phosphate. The difference between the total phosphate and the inorganic phosphate equals the ester phosphate.

Reagents required

1. 10% trichloracetic acid
2. 60% perchloric acid
3. 5% ammonium molybdate
4. Amino-naphthol-sulphonic acid solution
   0.2 g. 1 : 2 : 4 : - amino-naphthol-sulphonic acid, 12 g. sodium metabisulphite, and 2.4 g. sodium sulphite dissolved in 100 ml. distilled water
   Keep in a dark bottle, and make fresh every two weeks
5. " Porous pot," small pieces about $\frac{1}{8}$" diameter.

The Standard Lovibond Comparator Disc 5/14

The disc covers the range 0.02 mg. to 0.16 mg. of Phosphate (as P).

Technique for blood

Inorganic Phosphate

Add 2 ml. of freshly-drawn blood or oxalted plasma to 8 ml. of 10% trichloracetic acid, shake and filter. Place 5 ml. of filtrate (equivalent of 1 ml. of blood or plasma) in a 10 ml. test-tube, and add 0.7 ml. perchloric acid, 0.7 ml. ammonium molybdate, and 0.5 ml. amino-naphthol-sulphonic acid solution, and finally distilled water to the 10 ml. mark. Mix, and leave for 10 minutes for the colour to develop. At the end of this time, place the solution in a comparator test tube (or 13.5 mm. cell) in the right-hand compartment of the comparator.

* All values are expressed in terms of the element, P.

and a " blank " of distilled water in the left-hand compartment so that it comes behind the colour standards in the disc. Hold the comparator facing a uniform source of white light — a north light when possible is best — and rotate the disc until the colour of the liquid is matched by one of the glass standards. The value, expressed as mg. of phosphorus in the test solution (i.e. in 1 ml. of the blood or plasma), is then read off from the indicator window at the bottom right-hand corner of the comparator. Multiplied by 100, this result gives mg. inorganic phosphate (as P) per 100 ml. of blood or plasma.

Total Phosphate

Place 5 ml. of the trichloracetic acid filtrate from plasma, or 0.5 ml. of the filtrate from whole blood, in a test tube, add 1 c.c. of perchloric acid and a small piece of porous pot. Heat the tube carefully with a micro-burner or on an electric heater at such a rate that the liquid boils gently. When this has evaporated to a small volume the solution turns black or brown, and white fumes of perchloric acid are evolved. Continue the heating until the hot mixture turns yellow and then colourless. Remove from heater and allow to cool, then dilute to about 5 ml. with distilled water. Add 0.7 ml. ammonium molybdate, 0.5 ml. amino naphthol sulphonic acid solution, and distilled water to 10 ml. The solution is then transferred to the comparator test-tube or 13.5 mm. cell and the reading taken as above described.

When plasma is used, the result is multiplied by 100, and when whole bood is used the result is multiplied by 1,000 to obtain mg. per 100 ml. of total trichloracetic acid-soluble phosphate. Ester phosphate = total phosphate — inorganic phosphate.

Technique for Inorganic Phosphate in Urine

Introduction

The amount of inorganic phosphate excreted by persons on a normal diet varies between 1.5 and 2 g. (as P) per 24 hours.

In " balance " experiments where a knowledge of the intake and output of calcium and phosphate is of value, the excretion of phosphate is measured.

Technique

Accurately measure 0.2 ml. of urine into a test tube. Add water, perchloric acid, molybdate and amino-napthol-sulphonic acid solution as in the estimation of inorganic phosphate in the trichloracetic acid filtrate from blood.

The figure shown at the indicator window gives the mg. P contained in the measured volume of urine. If the colour is too dark a lesser volume of urine is taken.

Note

Details of the modification for water used in steam generation will be supplied by The Tintometer Ltd. on request:

References

1. C. H. Fiske and Y. Subbarrow, *J. Biol. Chem.*, 1925, **66**, 375
2. H. D. Kay, *J. Biol. Chem.*, 1932, **99**, 85
3. E. J. King, *Biochem. J.*, 1932, **26** 292
4. G. Stearns and E. Warweg, *Amer. J. Diseases of Children*, 1935, **49**, 79

The Determination of Phosphate (4)

using hydroquinone and ammonium molybdate

Introduction

The measurement of small amounts of phosphate is called for in dealing with a boiler water which is being treated by a phosphate conditioning system. A daily determination of soluble phosphate concentration is recommended by most authorities, in order that optimum conditions may be maintained. The details of technique should be carefully followed, as departures therefrom have been shown to cause inaccuracies.

Owing to the various, and sometimes unexpected, interfering substances and conditions which may be encountered in boiler waters, it must be stated that no method of estimation can be guaranteed always to give precisely accurate readings under all conditions, but it is claimed that by following the technique herein described, results may always be relied upon as accurate to within a tolerance of $\pm$ 5 parts per million, and in the majority of cases less than this. This method is also useful in estimating phosphate in soils and plants.

Principle of the method

The test consists of adding ammonium molybdate to an acid solution of the phosphate. The ammonium phosphomolybdate formed is then reduced to a lower state of oxidation resulting in a blue-coloured compound said to have the composition $(MoO_2.4MoO_3)_2.H_3PO_4$. The reducing agent used in this method is hydroquinone.

Reagents required

1. Sulphuric acid solution

   To about 500 ml. of distilled water add 65 ml. of sulphuric acid (analytical reagent grade) and dilute the mixture to exactly 1 litre.

2. Acid—molybdate solution

   Dissolve ***without heating*** 8.8 g. of ammonium molybdate (analytical reagent grade) in about 100 ml. of distilled water.

   To about 300 ml. of distilled water add 38 ml. of Sulphuric Acid (analytical reagent grade). Add the diluted acid to the ammonium molybdate solution and dilute the mixture to exactly 500 ml.

3. Hydroquinone solution

Hydroquinone (pure)	5 g.
Distilled water	500 ml.
Dissolve ***completely*** and then add sulphuric acid, concentrated, (analytical reagent grade)	0.3 ml.

   This solution slowly darkens in colour, but will keep for about 3 weeks in an amber glass bottle in the dark

4. Carbonate sulphite

   Dissolve in 500 ml. of distilled water 130 g. of anhydrous potassium carbonate (analytical reagent grade) and 24 g. of sodium sulphite (analytical reagent grade) $(Na_2SO_3.7H_2O)$.

The Standard Lovibond Comparator Disc 3/12

The disc covers the range 0—80 mg. per litre (=parts per million) of phosphate, calculated as PO_4. The equivalent in terms of phosphorus (P) is 0 to 26.1 parts per million.

Technique

Filter the boiler water sample brilliantly clear, so that only soluble phosphate is determined. To 5 ml. of this filtrate, in a Nessler tube, add the reagents in the following order. During and after each addition, swirl the sample to mix thoroughly :—

2 ml. molybdate solution (reagent 2).
1 ml. hydroquinone solution (reagent 3).

The solution should preferably be kept at a temperature of 25° C. ± 2°. Allow to stand 5 minutes, to develop the green phosphate colour. During this time, measure 2 ml. of the carbonate-sulphite solution (reagent 4) into a separate Nessler tube. At the end of the 5 minutes quickly pour the solution under test into the 2 ml. of carbonate-sulphite solution, pouring backwards and forwards a few times to ensure thorough mixing. Transfer to the test tube supplied, and place in the right-hand aperture of the comparator. A "blank" sample is prepared at the same time and in the same manner, substituting 2 ml. of the sulphuric acid solution (reagent 1) for the 2 ml. of molybdate solution. This is placed in a test tube in the left-hand aperture of the comparator, and compensates for any inherent colour in the boiler water or any slight colour of the hydroquinone solution.

Hold the comparator facing a uniform source of white light—a north window whenever possible is best—and compare the colour produced in the test solution with the colours in the standard disc, rotating the latter until a colour match is obtained. If the colour produced is deeper than the disc colours, the test should be repeated using a smaller volume of the boiler filtrate and adding distilled water to give a total volume of 5 ml. The degree of dilution must be allowed for when calculating the final answer.

The blue colour of the solution fades, so comparison should be made immediately the colour has been developed. Bubbles in the solution which cling to the side of the tube render matching difficult, and should be removed by gently tapping the tube.

The figures shown at the indicator window represent mg. per litre (=parts per million) of PO_4 in the boiler water, when 5 ml. of the sample is taken for the test.

Notes

1. This test has been found to be very satisfactory for many solutions other than boiler waters. For example, W. Plant, J. O. Jones, and D. J. Nicholas (Long Ashton Research Station, Bristol University, Annual Report 1944) have successfully applied it to extracts of plant tissues.

2. This modification of a well-known method was evolved by the chemical staff of I.C.I. (Alfloc) Ltd., to whom acknowledgement is made for advice and assistance.

3. For details of a special kit, incorporating this test, for the assessment of mineral deficiencies in plants, apply to The Tintometer Ltd., Salisbury.

The Determination of Phosphate (5)

using a simplified vanadate / molybdate method

Introduction

This is an extremely simple method for routine tests away from the laboratory, and has been used for the determination of phosphorus in iron and steel[1, 2].

Principle of the method

The method is based on the reaction of phosphates with molybdates in the presence of vanadate to form yellow phosphovanadio molybdate. In steel analysis the phosphorus is oxidised to phosphate by potassium permanganate, in nitric acid solution.

Reagents required

(1) 0.25% ammonium vanadate (2.5 g. ammonium vanadate dissolved in water, 20 mls. of concentrated nitric acid added and made up to 1 litre)

(2) 5% ammonium molybdate, aqueous solution

The Standard Lovibond Comparator Disc 3/38

The disc covers the range of 10 to 100 parts per million (mg. per litre) phosphate calculated as PO_4.

Technique

Place 5 ml. of the solution to be tested in a Lovibond Comparator test tube, add 1 ml. of solution (1) and 1 ml. of solution (2). Make up to the 10 ml. mark with distilled water, mix, and place in the right-hand compartment of the comparator. In the left-hand compartment, behind the glass colour standards, place a " blank " of 1 ml. of reagent (1) made up to 10 ml. with the untreated sample. Match after exactly 15 minutes, holding the comparator facing a uniform source of white light. The figure read from the indicator window represents parts per million PO_4.

Note

The colour produced differs slightly when testing waters of neutral reaction as compared with water which are acid, but it is always possible to identify the appropriate step in the scale.

References

1. R. Schroder, *Stahl u. Eisen*, 1918, **38**, 316
2. G. Misson, *Chem. Zeit.*, 1922, **32**, 633, *Ann. chim. anal. chim. appl.*, 1922, **4**, 267

The Determination of Phosphate (6)

using ascorbic acid and ammonium molybdate

Introduction

This[1] modification of Deniges' method[2] for the determination of phosphate is claimed to have the advantages of colour stability, and less dependance on the sample of ammonium molybdate used, than previous modifications[3,4]. It is also relatively insensitive to interference by other ions (Note 1) particularly iron. It has been found to be especially suitable for the estimation of phosphate in boiler-feed and -blowdown waters.

Principle of the method

The method depends, as do many previous methods, on the reduction of molybdophosphate to molybdenum blue. The reduction is carried out by ascorbic acid, at the boiling point of the solution, and the colour so formed is compared with permanent Lovibond glass standards.

Reagents required

1. Ammonium molybdate (analytical reagent grade) 10g.
   Distilled water to produce 100 ml.
   When dissolved, add slowly to a cooled mixture of
   Sulphuric acid (analytical reagent grade) 150 ml.
   Distilled water 150 ml.
   This reagent will keep indefinitely if stored in a boro-silicate glass bottle, and protected from light.
2. Ascorbic acid
3. Sulphuric acid —*N* solution
   Add 49g. of sulphuric acid (analytical reagent grade), slowly to 500 ml. of distilled water while cooling. When cold make up to 1 litre.

The Standard Lovibond Comparator Disc 3/51

This disc covers the range 10—400 μg. of phosphate (calculated as PO_4), in 9 steps.

Technique

Measure 20 ml. of the sample (Note 2) into a 100 ml. calibrated flask. Add sufficient *N* sulphuric acid (reagent 3) to neutralise the alkalinity and dilute to 100 ml. with distilled water. Measure 25 ml. of this solution into a 100 ml. beaker, add 15 ml. of distilled water, and 4 ml. of ammonium molybdate solution (reagent 1) and mix. Add 0.1g. ascrobic acid, cover with a watch glass, heat to the boiling point, and boil gently for 1 minute only. Cool the solution quickly, transfer to a 50 ml. flask, and dilute to 50 ml. with distilled water.

Carry out a blank test on the reagents with distilled water in the place of the sample. Transfer a portion of the test solution to a standard test tube and place in the right-hand compartment of the Lovibond Comparator. Transfer a portion of the blank solution to an identical test tube and place this in the left-hand compartment. Compare the colour of the test solution with the colours of the permanent glass standards in the disc using north daylight wherever possible.

Notes

1. Up to 1,000 p.p.m. of iron, chloride equivalent to 10% of sodium chloride, nitrate equivalent 2,500 p.p.m. of sodium nitrate and sulphate and perchlorate equivalent to at least 25% of the equivalent sodium salt are claimed[1] to have no effect on this determination.

The presence of 2,500 p.p.m. of soluble silica can also be tolerated.

2. For the determination of phosphate present in boiler-feed water as hexametaphosphate, it is recommended[1] that the hexametaphosphate be hydrolysed by neutralisation of the sample, addition of 1 ml. of hydrochloric acid (sp. gr. 1.18) and evaporation of the solution to dryness.

References

1. D. N. Fogg and N. T. Wilkinson, *Analyst*, 1958, **83,** 406
2. G. Denigès, *Compt. Rend.*, 1920, **171,** 802
3. W. M. Holman and A. G. Pollard, *J. Soc. Chem. Ind.*, 1937, **56,** 339T
4. The Determination of Phosphate (1), "*Colorimetric Chemical Analytical Methods*," The Tintometer Ltd., Salisbury, England

The Determination of Phosphate (7)

Single-reagent method

using vanadomolybdate reagent

Introduction

This method is a modification of Phosphate (5) using a single mixed reagent. This new reagent is stable for at least twelve months, is lighter in colour than the original mixture of two reagents, which results in more accurate differentiation of successive steps in the disc with a consequent increase in accuracy, and the final colour developed in the presence of phosphate is more stable. These improvements have been found to make this test especially suitable for the control of phosphate treatment of boiler-feed water.

Principle of the method

In the presence of vanadates, phosphates react with molybdates to form yellow phospho-vanadomolybdate. The intensity of this yellow colour, which is proportional to the amount of phosphate present, is determined by comparison with a series of Lovibond permanent glass standards. If the boiler water is coloured by the presence of organic matter, this is removed before the test by treatment with a suitable oxidising agent.

Reagents required

1. ***Vanadomolybdate reagent***

Solution A: 20 g. of ammonium molybdate tetrahydrate dissolved in 250 ml. distilled water
Solution B: 1 g. ammonium metavanadate dissolved in 40 ml. nitric acid (sp. gr. 1.42) and 200 ml. distilled water

Mix solutions A and B, add 100 ml. nitric acid (sp. gr. 1.42) and dilute to 1,000 ml. with water.

2. ***Oxidising mixture***

Intimately mix, by grinding in a mortar, 100 g. of potassium persulphate and 60 g. of sodium carbonate, and sieve through a 40 mesh sieve.

The Standard Lovibond Comparator Disc 3/60

This disc covers the range 10 to 100 p.p.m. of phosphate, calculated as PO_4, in 9 steps.

For control of small low-pressure boilers where accurate determination of phosphate reserve is not required, a simplified Lovibond Block Comparator is available, containing two colour standards only. These correspond to 30 p.p.m. (low) and 80 p.p.m. (high) of phosphate respectively.

Technique

Filter the water through a No. 42 Whatman filter paper into a 50 ml. stoppered measuring cylinder. Use the first few ml. of filtrate to rinse out the cylinder. Filter between 20 and 25 ml. of water, and then add an equal volume of reagent 1. Stopper the cylinder and mix the contents. Rinse one of the comparator 13.5 mm. tubes or cells with this solution, then fill the cell and place it in the right-hand compartment of the comparator. In the left-hand compartment place a blank prepared by mixing equal volumes of reagent 1 and distilled water. Allow the colour to develop for at least three minutes and then match the colour of the sample tube against that of the standards in the disc, using north daylight if possible. If the colour is deeper than the 100 p.p.m. colour standard the solution may be diluted with distilled water and due allowance made for the degree of dilution.

If the original water sample is coloured, measure 50 ml. into a 150 ml. beaker, add 1-2 g. of reagent 2, boil until colourless, cool and make up to 50 ml. with distilled water. Filter through No. 42 Whatman filter paper and proceed as above.

Note

This test has been developed in collaboration with the Water Treatment Section, Technical Service Department of Albright and Wilson (Mfg.) Ltd.

The Determination of Silica (1)

using ammonium molybdate

Introduction

Silica is present in most waters, larger concentrations usually being found in well water derived from chalk soils. The presence of silica in river water is often an indication of the ability of the water to promote the growth of certain organisms, such as diatoms, which require silica for their growth. Silica is also added in certain water treatments to assist the floc formation where coagulation is used.

This test is also used on airfields, because a limit of 3 ppm SiO_2 is usually placed on the silica content of de-ionised water used on aircraft to increase the thrust of turbo-jet engines during take-off.

Principle of the method

The colour standards are designed to match the colours produced by the addition of ammonium molybdate and sulphuric acid to solutions containing silica. In this test the concentration of the sulphuric acid present in the mixture is important, and the presence of either too little or too much free acid results in diminution of the intensity of the yellow colour due to silica. It is essential therefore, when carrying out the test, to adhere strictly to the conditions described below, under which the colour glasses have been standardised. In particular, the test **must** be carried out within the stated temperature range.

Reagent required

A 10 per cent w/v aqueous solution of ammonium molybdate (analytical reagent grade)
2N sulphuric acid
Mix 1 volume of the ammonium molybdate solution with 2 volumes of the sulphuric acid.

The Standard Lovibond Discs, Comparator Disc 3/13 and Nessleriser Disc NN

Disc 3/13 covers the range from 2.5 to 25.0 parts per million of silica, calculated as SiO_2, and is designed for use with the Special-Purposes Comparator and a 4 cm. cell.

Disc NN covers the range from 50γ to 1,000γ (0.05 to 1.0 mg.) of silica calculated as SiO_2.

Technique

(a) ***Comparator***

Fill one of the 4 cm. glass cells with distilled water and place in the left-hand compartment of the comparator. If the solution under test is not colourless, the cell should be filled with the solution instead of with distilled water. In a suitable flask place 25 ml. of the solution under examination, at 25°—35°C*., add 3 ml. of the reagent, mix, allow to stand for 10 minutes and then pour into the other 4 cm. cell in the right-hand compartment of the instrument. Hold the comparator facing a uniform source of white light — a north window wherever possible is the best — and compare the colour produced with the colours in the standard disc, rotating the latter until a colour match is obtained. Should the colour in the test solution be deeper than the standard colour glasses, a fresh test should be carried out using a smaller quantity of the solution under examination and diluting to 25 ml. with distilled water before adding the reagents, due correction being made to the answer obtained. The figures on the disc represent parts per million of silica when 25 ml. of the solution are taken for the test.

(b) ***Nessleriser***

Fill one of the Nessleriser glasses to the 50 ml. mark with distilled water and place in the left-hand compartment of the Nessleriser. If the solution under test is not colourless, the Nessleriser glass should be filled with the solution instead of with distilled water. Fill the other Nessleriser glass to the mark with the solution under examination at 25°—35° C.,* add 6 ml. of the reagent, mix, allow to stand for ten minutes and then place in the right-hand compartment of the instrument. Stand the Nessleriser before a uniform source of white light — a north window wherever possible is the best — and compare the colour produced with the colours in the standard disc, rotating the disc until a colour match is obtained. Should the

*It is important that the test should be carried out at the temperature stated.

colour in the test solution be deeper than the standard colour glasses, a fresh test should be carried out using a smaller quantity of the solution under examination and diluting to 50 ml. with distilled water before adding the reagents.

Notes

1. Most colourless salts, even when present in relatively large quantities, are without influence upon the colour produced in the test, provided the concentration of free acid is not unduly disturbed. Phosphates, however, must be absent, since they respond to the test and yield a yellow colour similar to that produced by silica.

2. Phosphates may be removed by treating 50 ml. of the solution under test with 25 ml. of Sörensen's borate buffer solution *p*H 10 and 1 ml. of 2N calcium chloride solution ; after mixing and allowing to stand for 2 hours the mixture is filtered. The test is made on 25 ml. of the filtrate by the method described above. As Sörensen's borate buffer invariably contains silica derived from the glass bottle, a blank test should be carried out and the necessary correction made. The result must be multiplied by 1.5.

3. It must be emphasized that the readings obtained by means of the B.D.H. Lovibond Nessleriser and disc are only accurate provided that Nessleriser glasses are used which conform to the specification employed when the discs were being calibrated, namely that the 50 ml. calibration mark shall fall at a height of 113 mm. plus or minus 3 mm. measured internally.

The Determination of Silica (2)

using 1-amino-2-naphthol-4 sulphonic acid

Introduction

This test, due to W. E. Bunting, is designed primarily to measure the soluble silica content of boiler feed water for modern high pressure steam turbines.

Principle of the method

The standard glasses match the molybdenum blue colour produced by the reduction of silico-molybdate with 1-amino-2-naphthol-4-sulphonic acid in sulphuric acid solution. The addition of tartaric acid avoids interference from phosphates.

Reagents required

1. Sulphuric acid-molybdate reagent
   To 75 g. of ammonium molybdate (analytical reagent grade) dissolved in silica-free distilled water, add 322 ml. of 10N sulphuric acid and make up to 1 litre.
2. Tartaric acid reagent
   Dissolve 10 g. of tartaric acid (analytical reagent grade) in 100 ml. of silica-free distilled water.
3. 1-Amino-2-napthol-4-sulphonic acid reagent
   (a) Dissolve 90 g. of sodium metabisulphite in 800 ml. of silica-free distilled water.
   (b) Dissolve 14 g. of sodium sulphite hydrated (analytical reagent grade) in approximately 100 ml. of silica-free distilled water.
   To solution (b) add 1.5 g. of 1-amino-2-napthol-4-sulphonic acid, mix until dissolved and add to solution (a). Make up total volume to 1 litre.

The Standard B.D.H. Lovibond Nessleriser Disc NV

The disc covers the range 0.2-1.0 p.p.m. SiO_2.

Technique

To a 50 ml. sample at a temperature between 20° and 30° C. in a Nessleriser glass add 2 ml. of sulphuric acid-molybdate reagent. After five minutes, add 4 ml. of tartaric acid reagent and 1 ml. of 1-amino-2-naphthol-4-sulphonic acid reagent. Mix and allow to stand for twenty minutes, making quite certain that the temperature does not fall below 20° C. Place the Nessleriser glass in the right-hand compartment of the Nessleriser and rotate the disc until a match is obtained.

Notes

1. It is important that the test should be carried out at between 20° and 30° C., since it has been found that at temperatures under 20° C. the reaction does not proceed to completion and low results are obtained.

2. It must be emphasized that the readings obtained by means of the B.D.H. Lovibond Nessleriser and disc are only accurate provided that Nessleriser glasses are used which conform to the specification employed when the discs were calibrated, namely that the 50 ml. calibration mark shall fall at a height of 113 mm. ± 3 mm. measured internally.

Reference

W. E. Bunting, *Ind. Eng. Chem., (Anal. Ed.)*, 1944, **16**, 612

The Determination of Silver

using sodium sulphide

Introduction

This test has been produced in collaboration with Ilford Ltd., of Ilford, Essex, in order that the concentration of silver present in used photographic fixing baths may be quickly ascertained. This is important in a busy processing laboratory where large quantities of films are processed in large fixing baths and the concentration of silver builds up to a point where the recovery of the silver is a commercial proposition. Ilford Ltd. have produced a Silver Recovery Unit based on an electrolytic method, and it is necessary to know the concentration of silver in the solution, as the current applied and the time taken is dependent upon that information: further if the recovery operation is continued below a certain silver concentration, the fixer will break down through " sulphiding " and be ruined.

Principle of the method

This test is based on the production of colloidal silver sulphide in a solution buffered by citrate. The colloid is protected by gelatin and its colour compared with Lovibond permanent glass standards.

Reagents required

1.	Citric acid	9 g.
	Sodium citrate, tribasic	100 g.
	Distilled water	to 1 litre
2.	Gelatin	4 g.
	Phenol	10 g.
	Distilled water	to 1 litre
3.	Sodium sulphide (analytical reagent grade)	80 g.
	Sodium sulphite, anhydrous	50 g.
	Distilled water	to 1 litre

These solutions may be obtained, ready prepared, from Ilford Ltd., Ilford, Essex.

The Standard Lovibond Comparator Disc 3/41

The disc covers the range 2 to 10 grams per litre in eight colour steps.

Technique

Pour 5 ml. of solution 1 and 5 ml. of solution 2 into a 50 ml. graduated cylinder. Add to this 1 ml. of the fixing solution to be tested. Make up to 50 ml. with water (tap water will do) and mix well. Pour 10 ml. of the above mixture into one of the 10 ml. calibrated Lovibond test tubes and place in the left-hand compartment of the comparator, to come behind the coloured glass standards and act as a " blank " for inherent colour. To the remaining 40 ml. in the cylinder add 1 ml. of solution 3, mix well and stand for 5 minutes. Fill the other test tube with this solution and place in the right-hand compartment of the comparator.

Hold the comparator facing a uniform source of white light, preferably a North window and compare the colour of the solution with the colours in the disc, rotating the disc until a colour match is obtained. The value read from the bottom right-hand aperture of the comparator face indicates grams of silver per litre of solution.

Always carefully wash the tubes and pipettes between tests.

Note

If the solution produced by the addition of solution 3 shows a brown gelatinous deposit, this indicates that solution 2 has deteriorated and must be replaced.

The Determination of Sodium

using manganous uranyl acetate

Introduction

This method, which is a modification of Woelfel's method,[1] was developed by Shawarbi and Pollard,[2] for the estimation of small amounts of sodium in soil solutions, extracts etc. The method was originally developed for use with the Lovibond-Schofield Tintometer, but has been adapted for use with the Lovibond Comparator.

Principle of the method

Sodium is precipitated as sodium manganese uranyl acetate, the precipitate dissolved in 2-N sulphuric acid and the manganese oxidised to permanganate with potassium periodate. The permanganate colour is then compared with Lovibond permanent glass standards either using the Tintometer or the Comparator and a standard disc.

Reagents required

1. Manganous uranyl acetate stock solution :—

Uranyl acetate	160 g.
Manganous acetate	490 g.
30% acetic acid	138 ml.
Distilled water	1500 ml.

After the chemicals have dissolved the solution is made up to 2 litres, allowed to stand for 24 hours, filtered and stored in a dark bottle.

2. Dilute alcoholic manganous uranyl acetate solution :—

Reagent 1	180 ml.
95% alcohol	60 ml.

Mix, stand for 4 hours, filter through No. 42 Whatman filter-paper. This solution is stable for 4 weeks if stored in a dark bottle.

3. Zinc uranyl acetate stock solution :—

Uranyl acetate	160 g.
Zinc acetate	440 g.
30% acetic acid	138 ml.
Distilled water	1500 ml.

After the chemicals have dissolved the solution is made up to 2 litres, allowed to stand for 24 hours, filtered and stored in a dark bottle.

4. Dilute alcoholic zinc uranyl acetate wash solution :—

Reagent 3	120 ml.
95% alcohol	40 ml.

This solution is placed in an ice-bath for 1 hour and then saturated with 32 mg. of solid manganous triple salt, prepared as described below, and, after at least an hour, is filtered through a No. 4 sintered glass crucible using suction.

This reagent remains serviceable for three weeks if stored in a dark bottle.

Sodium manganous uranyl acetate salt is prepared by treating 125 ml. of Reagent 1 with 2 ml. of a 5% sodium chloride solution. After 30 minutes the liquid is centrifuged. The supernatant liquid is removed and the precipitate washed 3 times with 95% alcohol, twice with ether, and dried at ambient temperature.

5. Oxidising solution :—

Potassium periodate	0.75 g.
Distilled water	150 ml.
Phosphoric acid (syrupy)	25 ml.

The periodate is dissolved in the water, the acid added, and the solution diluted to 200 ml. This solution is stable for 4 weeks in a cool place.

The Standard Lovibond Comparator Disc 3/42

The disc covers the range 50 p.p.m. to 250 p.p.m. of sodium (Na), and must be used in conjunction with a dulling screen.

Technique

1 ml. of the test solution, containing 50—500 p.p.m. of sodium (50—250 p.p.m. if a Lovibond Comparator is being used) is placed in a 30 ml. beaker. 10 ml. of reagent 2 are added and the solutions are mixed. After at least 4 hours at room temperature the precipitate is collected on a No. 4 sintered glass crucible, using suction. The beaker and the precipitate are washed five times, using 5 ml. of ice-cold wash liquid (Reagent 4) each time. The outside of the crucible is washed with water. The triple acetate salt is dissolved by adding three successive portions of 5 ml. of warm 2N sulphuric acid, using slight suction. The crucible is washed twice with distilled water. The filtrate and washings are collected in a 100 ml. beaker, the alcohol is removed by boiling on a low flame for 5—7 minutes and 25 ml. of Reagent 5 added. Boiling is continued for a further 5—7 minutes to ensure maximum colour development. The coloured solution is cooled, and made up to 50 ml. in a graduated flask.

The colour obtained is matched against permanent glass standards either by using the Tintometer and a 1 cm. cell, in which case a calibration curve must be constructed using known amounts of sodium, or by means of the comparator and a standard 13.5 mm. tube or cell.

Notes

1. The common soil constituents Ca, Mg, Fe, Al, Mn, and phosphate do not interfere even when present in quantities far in excess of any likely to be met with in soil solutions and extracts. Unlike the original Woelfel method[1] this method does not neceessitate the removal of phosphate.

2. Potassium does interfere, giving high results. The interference is only serious when the potassium concentration exceeds three times the sodium concentration.

3. For use with solutions containing less than 50 p.p.m. 10 ml. of the test solution are taken and reduced to 1 ml. by evaporation before proceeding with the above technique.

References

1. W. C. Woelfel, *J. Biol. Chem.*, 1938, **125,** 219
2. M. Y. Shawarbi and A. G. Pollard, *J. Soc. Chem. Ind.*, 1943, **62,** 71

The Determination of Sulphate

using barium chloranilate

Introduction

Sulphur is an essential element for plant growth and is obtained from the soil in the form of sulphate ions. The estimation of available sulphate in soils is therefore a necessary part of agricultural and horticultural control analysis. There is a requirement for a method of determining sulphate which is equally suitable for use in the field or in the laboratory. This adaptation of the chloranilic acid method[1] for the determination of sulphate has been developed[2] to meet this need.

Although this method was developed especially for soil analysis it is applicable to the determination of sulphate in water, or in aqueous extracts of materials other than soils, ***provided that the pH of the solution is kept within the stated limits of 3.0 to 3.5.***

Principle of the method

An extract of the soil is prepared by the standard Morgan technique[3], and excess soil bases are removed by means of an ion exchange resin. The chloranilic acid liberated from barium chloranilate by a portion of the extract, under conditions of controlled *p*H, is estimated by comparison of the colour produced against the colour of a range of Lovibond permanent glass standards.

Reagents required

1. Barium chloranilate
2. Exchange resin Zeo-carb 225 (H form) 50–60 mesh
3. Ethyl alcohol—absolute
4. Potassium hydrogen phthalate, 0.05**M.** (*p*H 4.0 buffer solution)
5. Wide-range *p*H test papers

The Standard Lovibond Comparator Disc 3/49

The disc covers the range 5 to 80 p.p.m. of sulphate (SO_4^{-2}) and must be used with the dulling screen provided.

Technique

Pass the Morgan soil extract through a column (20 × 1 cm.) of the ion exchange resin, and test the percolate with the *p*H test papers. When the *p*H of the percolate falls to about 2.0 as indicated by a change from orange to pink on the test paper, collect 5 ml. of percolate for analysis. Transfer 2 ml. of this percolate to a centrifuge tube together with 1 ml. of phthalate solution, 2 ml. of water and 5 ml. of ethyl alcohol. Mix, add approximately 0.03g. of barium chloranilate, shake the mixture for 5 minutes and then centrifuge at 2,500 r.p.m. for 5 minutes.

Transfer the supernatant liquid to a standard test tube and place this in the right hand compartment of the Comparator. Fill an identical tube with water and place in the left-hand compartment. Compare the colour of the test solution with the permanent glass standards in the disc, using north daylight wherever possible.

Notes

1. Morgan's reagent, used in extracting soil samples, is prepared as follows :—
   100 grams sodium acetate tri-hydrate, analytical reagent grade
   30 ml. glacial acetic acid
   in 1 litre distilled water.

2. The potassium hydrogen phthalate is added to the percolate (which has a *p*H of approximately 2.0) ***to bring it to a final value between 3.0 and 3.5 pH, which value is necessary in order to produce the correct colours in the test.*** The presence of the Morgan's extractant also influences the test colours.

3. 0.03 g. of barium chloranilate is conveniently measured by a spatula made of a piece of glass rod of suitable size, which may be found by experiment.

4. Small hand-operated centrifuges for field use are readily available, and may be obtained from The Tintometer Ltd.

References

1. J. Bertolacini and J. E. Barney, *Anal. Chem.*, 1957, **29,** 281
2. W. Y. Magar and A. G. Pollard, *Chemistry & Industry,* 1961, No, **16,** April, 505
3. M. F. Morgan, "*Chemical Soil Diagnosis by the Universal Soil Testing System,*" Bull. Conn. Agric. Expt. Sta., 1941, **372**

The Determination of Sulphite

using potassium iodide-iodate

Introduction

Sulphites are encountered in industrial wastes, in boiler water, and in water exposed to atmospheres containing sulphur dioxide. Solutions of sulphur dioxide are also used in certain bleaching processes, and in preserving foodstuffs.

Principle of the method

The test is based on the quantitative reaction between sulphites and excess iodine in acid conditions, and the standards are matched against the residual colour obtained when a constant amount of iodine is reacted with different amounts of sulphite. All substances reacting with iodine under the conditions of the test will interfere, and hence *e.g.* nitrates and sulphides must be absent.

Reagents required

1. Potassium iodide solution N/10 (16.6 g. KI per litre)
2. Potassium iodate solution N/40 (0.892 g. KIO_3 per litre)
3. Hydrochloric acid, analytical reagent grade, (wt. per ml. at 20°C. 1.18 g.)

The Standard B.D.H. Lovibond Nessleriser Disc NOB

The disc covers the range from 2 to 50 parts per million Na_2SO_3 using a 25 ml. sample (25 to 625γ SO_2). A dulling screen is required.

Technique

1 ml. of potassium iodate solution, 1 ml. of potassium iodide solution and 0.5 ml. of hydrochloric acid are placed in a Nessleriser glass in that order ; 25 ml. of the sample are then added, and, after mixing, the volume is adjusted to 50 ml. with distilled water. The colour obtained is matched against the disc after standing for 15 minutes.

Note

It must be emphasized that the readings obtained by means of the B.D.H. Lovibond Nessleriser are only accurate provided that Nessleriser glasses are used which conform to the specification employed when the discs were calibrated, namely that the 50 ml. calibration mark shall fall at a height of 113 mm. $\pm$ 3 mm. measured internally.

The Determination of Sulphur

using lead acetate

Introduction

This disc has been produced in collaboration with the Technical Department of the National Benzole Company and is specified in the S.T.P.T.C. Method, Serial L.B.20. The details of the technique are published with the permission of that Committee.

Principle of the method

A strip of clean copper foil is immersed in the sample under examination and held for 2 hours at 50° C. The stain (if any) of sulphide deposited on the copper is converted into hydrogen sulphide, and this is estimated colorimetrically by means of lead acetate paper.

The Standard Lovibond Comparator Disc 3/22

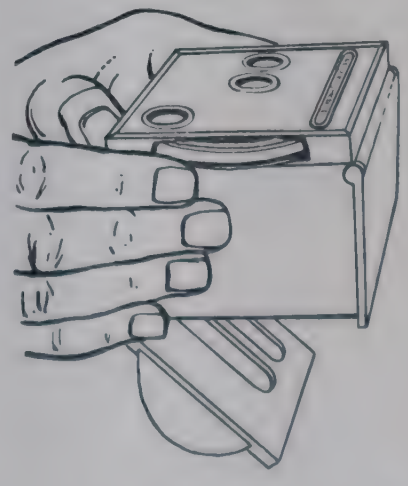
The disc covers the range 1 to 8 micrograms (millionths of a gram) of sulphur. This disc is designed for use with the Special Purposes Comparator.

Technique

For full details for producing the stain on a copper strip, reference should be made to the S.T.P.T.C. Handbook, " Standard Methods for Testing Tar and its Products."

The test papers are prepared by soaking No. 1 Whatman filter paper for 1 minute in a solution of 10 grams lead acetate in 90 ml. distilled water, to which are added 5 ml. of glacial acetic acid and 10 ml. pure glycerol. The superflous liquid is allowed to drain off and the papers are dried in an oven at 90°—100° C. until they are just dry (10—15 minutes). Such a paper is placed in position in an aluminium holder in a streaming apparatus, (see reference), and pure hydrogen gas streamed through for 10 minutes to clear out all other gas. Hydrochloric acid 18% w/w is then allowed to react with the stained copper strip, and the gas stream continued for 30 minutes, to ensure that all the hydrogen sulphide produced is swept through the test paper.

The Special-Purposes Lovibond Comparator is made ready with all the internal fittings removed, and the back flap fastened in position by the quadrant hinge. A clean blank of similar filter paper is placed behind both fields, and held in position by means of the opal glass and clip. The stained paper is removed from the apparatus with tweezers, allowed to dry for one minute, and then placed on the right-hand side of the flap on top of the clean filter paper. The appropriate disc is inserted in the lid of the comparator, the apparatus held about 18" from the eye, facing a good North light, so that both papers are evenly illuminated, and the disc rotated until a match is obtained, when the value is read from the indicator. A second paper is inserted in the hydrogen stream, and if any further stain is obtained, the value of this is added to the first result.



The sample of benzole is reported as causing a corrosion of the copper strip that will produce hydrogen sulphide equal to the number of micrograms of sulphur (as hydrogen sulphide) as read from the disc.

Notes

The test may also be employed to obtain a figure for the hydrogen sulphide present. A suitable volume of the benzole itself is inserted in the streaming apparatus and the gas streamed out by means of the hydrogen. The reading obtained represents the amount of hydrogen

sulphide actually present in the volume taken. If too great a concentration of hydrogen sulphide is present, the sample is diluted with pure benzole free from hydrogen suphide.

References

1. G. Claxton, and K. H. V. French, *J. Inst. Petroleum*, 1949, **35**, 496
2. S.T.P.T.C. *"Standard Methods for Testing Tar and its Products,"* 4th Edn. 1957
3. British Standard, 135 : 1953
4. British Standard, 458 : 1953

The Determination of Tin

using dithiol

Introduction

A method for the determination of small amounts of tin in biochemical materials and food is being increasingly called for by analysts, and there are a number of other occasions on which this test is required.

The method of Clark has been adapted from its previously published procedure using the Tintometer, so that through the use of a reagent which keeps the " lake " in suspension the matching may be now more easily performed in a Lovibond Comparator.

Principle of the method

In the presence of an acid solution of divalent tin, dithiol (1 : 2-dimercapto-4-methyl-benzene) forms a red lake. In this modification of the original test the tin is reduced to the divalent state by means of thioglycollic acid, and the red lake is held in suspension by sodium lauryl sulphate.

Reagents required

1. Hydrochloric acid (analytical reagent grade)
2. Thioglycollic acid (analytical reagent grade)
3. Sodium lauryl sulphate (sulphonated ' Lorol '), 1.0% w/v aqueous solution
4. Dithiol (1: 2-dimercapto-4-methyl-benzene), 0.125% w/v solution in 1.0% w/v aqueous sodium hydroxide, freshly made up as required

   (N.B.—This reagent seldom maintains its reactive strength for more than two days).

The Standard Lovibond Comparator Disc 3/35

The disc contains colour standards for 1, 2, 3, 4, 5, 6, 8, 10 and 12 parts per million of tin, present in the final 20 ml. of solution.

Technique

The preparation of the solution will of course depend upon the material under test, but food stuffs and biological material must first be ashed.

Place in a 20 ml. volumetric flask 10 ml. of the solution under test, which should be 0.5N in terms of hydrochloric acid and should contain not more than 0.24 mg. of tin. If a preliminary test shows that there is a higher concentration of tin present, a suitable dilution with 0.5N HCl should be carried out before a determination is undertaken.

Add to the solution in the flask in the following order:—

1 drop of thioglycollic acid
2.0 ml. of hydrochloric acid, concentrated
0.5 ml. of 1% sodium lauryl sulphate solution
1 ml. of dithiol reagent.

Mix thoroughly after the addition of each of these reagents. At the same time prepare a " blank " solution containing 10 ml. of 0.5N hydrochloric acid in place of the 10 ml. of test sample and the other reagents exactly as above.

Stopper the flasks and place in a water bath at 60°C for ten minutes. Cool to 20°C and make up the volumes in each case to the 20 ml. mark with distilled water. Place the test solution in a 13.5 mm. comparator cell or test tube in the right-hand compartment of the comparator and the " blank " in a similar cell or test tube in the left-hand compartment so that it comes behind the glass colour standards.

Hold the comparator facing a uniform source of white light—a north window whenever possible is best—and compare the colour produced in the test solution with the colours in the standard disc, rotating the latter until a colour match is obtained. The figure shown at the indicator window represents parts per million of tin in the final 20 ml. solution. Due correction must therefore be made to relate this to the amount of sample originally taken.

Note

The detection of tin is disturbed by the presence of copper nickel and bismuth, and less so by cobalt, since in this case tin is preferentially precipitated. Compounds produced by silvery mercury cadmium arsenic and lead are yellow, and therefore only slightly reduce the accuracy of the results. Nitrites and phosphates can interfere.

References

1. R. E. D. Clark, *Analyst*, 1936, **61**, 242 and
1937, **62**, 661
2. C. Kenyon and T. C. J. Ovenston, *Nature*, 1951, **167**, 727
3. T. C. J. Ovenston and C. Kenyon, *Analyst*, 1955, **80**, 566

The Determination of Titanium

using hydrogen peroxide

Introduction

This method may be used to determine the percentage of titanium, as TiO_2, in clays, rocks, minerals and ceramic materials.

Principle of the method

The test is based on the yellow colours produced by the reaction between titanium sulphate and hydrogen peroxide in acid solution.

Reagents required

1. Potassium pyrosulphate
2. Sulphuric acid, analytical reagent grade
3. Hydrogen peroxide (20 volumes, analytical reagent grade)

The Standard Lovibond Discs, Comparator disc 3/26, and Nessleriser discs NRA, NRB and NRC

Disc 3/26 covers the range 1.0 to 10 per cent. titanium (Ti). This disc is designed for use with a 5 mm. cell.

Disc NRA covers the range 0.025 to 0.225 per cent. of TiO_2.
Disc NRB covers the range 0.25 to 2.25 per cent. of TiO_2.
Disc NRC covers the range 0.05 to 0.2 per cent. of TiO_2.

The supplementary disc NRC is used only in conjunction with Disc NRB in order to obtain intermediate readings.

Technique

Ignite the Group III precipitate obtained from 1 g. of the sample by the usual methods for the analysis of a clay material, and fuse it in a platinum crucible with potassium pyrosulphate. Dissolve the cake in distilled water, add 20 to 30 ml. of sulphuric acid (reagent 2) dilute with distilled water to 250 ml. and mix thoroughly. To 50 ml. of this solution add 10 ml. of hydrogen peroxide (reagent 3), dilute with distilled water to 100 ml. and mix thoroughly.

Comparator

Pour into a 5 mm. cell. Place this cell in the right-hand compartment of the comparator, and leave the left-hand compartment blank. Hold the comparator facing a good source of north daylight, and rotate the disc until the nearest match is obtained. The figure shown in the indicator window represents percentage Ti in the original gram of sample taken.

Nessleriser

Transfer 30 ml. of this yellow solution to a Nessleriser glass and place it in the right-hand compartment of the Nessleriser.* In the other compartment of the Nessleriser place a Nessleriser glass containing 30 ml. of distilled water. Stand the Nessleriser before a uniform source of white light — a north window is best — and compare the colour of the test solution with the colours in one of the standard discs, rotating the disc until a colour match is obtained. The supplementary disc, which is placed on the top of the Nessleriser, should be used in conjunction with disc B for higher concentrations of TiO_2. The markings on the discs indicate the percentage of TiO_2, assuming that 1 g. of the sample has been taken for the test.

*If the original material contains more than 2.5 per cent. TiO_2 but not more than 4 per cent., 25 ml. of the original solution should be taken to make up to 100 ml. instead of 50 ml. The results obtained should be corrected accordingly.

Notes

1. The application of the B.D.H. Lovibond Nessleriser to the determination of titanium was suggested by Dr. G. R. Rigby of the British Refractories Research Association. The colour standards were prepared in the Laboratories of the Association.

2. It must be emphasised that the readings obtained with the B.D.H. Lovibond Nessleriser and disc are accurate only if Nessleriser glasses are used which conform with the specification used at the time the discs were calibrated, namely that the 50 ml. mark shall fall at a height of 113 ± 3 mm. measured internally.

Reference

A. Weller, *Ber.*, 1882, **15**, 2592

The Determination of Vanadium

using 8-hydroxyquinoline

Introduction

Vanadium is widely used as an alloying constituent in steels, where it imparts hardness and toughness. It must be separated from iron for the application of this test which was developed primarily for the examination of natural waters. The test may also be adapted to the estimation of vanadium in minerals and in plant ash.

Principle of the method

The procedure is based upon a qualitative test which consists in forming the reddish-brown, 8-hydroxyquinoline complex in dilute acetic acid solution, followed by extraction with amyl alcohol. For the purpose of the comparator disc, the published method has been modified (by the addition of sodium pyrophosphate), with a view to preventing the interference of iron.

Reagents required

1. A 5 per cent. w/v aqueous solution of sodium pyrophosphate
2. Glacial acetic acid (analytical reagent grade)
3. A 2.5 per cent. w/v solution of 8-hydroxyquinoline in a 10 per cent. aqueous solution of acetic acid
4. Amyl alcohol

The Standard Lovibond Comparator Disc 3/20

The disc covers the range from 10γ to 100γ (thousandths of a milligram) of Vanadium (V.) or 0.2 to 2.0 parts per million if a 50 ml. sample is taken.

Technique

To 50 ml. of solution to be tested, contained in a separator, add 10 ml. of a 5 per cent. aqueous solution of sodium pyrophosphate, followed by 0.2 ml. of glacial acetic acid, and 0.2 ml. of the 8-hydroxyquinoline reagent. Add 10 ml. of amyl alcohol, and shake vigorously for one minute. Allow to separate, reject the aqueous layer, and transfer the organic layer to a graduated cylinder. Dilute to 10 ml. with more amyl alcohol, and transfer to a 13.5 mm. cell or test tube and place in the right-hand compartment of the comparator. In the left-hand compartment place a similar cell containing distilled water, and, with the comparator facing a north window rotate the disc until a match is obtained. The figure shown indicates the amount of vanadium present in the 50 ml. of sample taken ; thus, if a colour equivalent to 50γ is produced, the amount of vanadium present is 0.05 mg. per 50 ml., or 1 part per million.

Notes

The amount of iron present in the test must not exceed 100γ since above this amount it cannot be effectively suppressed, and interferes by reducing the intensity of the vanadium colour and alters it shade. Fluorides, chlorides, bromides, iodides, sulphates, nitrates, nitrites and sulphites do not interfere, nor does the presence of moderate amounts of calcium, magnesium, sodium, potassium, manganese, lead or zinc. The test is disturbed by copper, when present in amounts comparable to the vanadium ; interference by titanium may be obviated by the addition of sodium fluoride, which prevents the formation of a colour by this element.

References

1. R. Montequi and M. Gallego, *Anal. Soc. Esp. Fisicoquim.*, 1934, **32**, 134
2. J. M. Bach and R. A. Trelles, *Anal. Asoc. Quim. Argentina*, 1940, **28**, 111

Part 4

Chemical Analysis

Organic

Summary of Tests in Part 4, and Equipment Required for Each Test

Determination of	Using	Disc No.	Screen	Cells	Instrument	Equipment required
Carbohydrates	Orcinol	3/8	—	13.5 mm.	Comparator	Pipettes (1, 10 ml.); water bath; heat; volumetric flask (50 ml.)
Carbon Disulphide	Diethylamine & copper acetate	3/18	—	40 mm.	Special-Purposes Comparator	Pipettes (1 ml.); stoppered measuring cylinders (25 ml.); water bath; heat; thermometer
Carbon Tetrachloride	Pyridine	3/44	—	13.5 mm.	Comparator	Pipettes (10, 20 ml.); volumetric flask (50 ml.); water bath; heat; running water
Carotene	—	3/23	—	25 mm.	Special-Purposes Comparator	Balance; water bath; heat; flask fitted with reflux condenser; filter funnel; filter papers; chromatographic column; Buchner flask; suction
Chloroform	Pyridine	3/45	—	13.5 mm.	Comparator	Pipettes (10, 20 ml.); volumetric flask (50 ml.); water bath; heat; running water
Cyanide	Pyridine-pyrazolone	NOC	—	50 ml.	Nessleriser	Pipettes (1, 5, 25, 250 ml.); volumetric flask (250 ml.); distillation apparatus; *p*H indicator papers; watch; heat; running water
D.D.T.	Nitric acid & alcoholic potash	3/34	Yes	5 mm.	Comparator	Balance; beaker (100 ml.); hot plate; chromatographic column pipettes (2, 3, 5, 10, 15 ml.); water bath; heat; separating funnels; glass wool; pump; volumetric flask (25 ml.)
Detergents	Methylene blue	3/24	Yes	13.5 mm.	Comparator	Pipettes (10 ml.); measuring cylinder (50 ml.); volumetric flask (50 ml.); separating funnel (100 ml.)
Dichlorophene	4-aminoantipyrene	3/57	—	5 mm.	Comparator	Balance; beaker; measuring cylinder (25 ml. and 100 ml.); Flask (volumetric 200 ml.); funnel and filter paper; Pipettes (1.5 ml. and 10 ml.); volumetric flask (50 ml.)
Formaldehyde	Chromotropic acid	NOD	—	50 ml.	Nessleriser	Pipette (4 ml.)
Furfuraldhyde	Aniline acetate	3/30	Yes	13.5 mm.	Comparator	Distillation apparatus; heat; running water; volumetric flask (250 ml.); Pipettes (0.5, 10 ml.)
Hydrazine	*p*-dimethylamino-benzaldehyde	NOH	—	50 ml.	Nessleriser	Pipettes (1, 10, 25 ml.)
Lauryl Pentachlor-phenol	Copper-pyridine reagent	3/37B	—	5 mm.	Comparator	Balance; distillation apparatus; measuring cylinder (250 ml.); separating funnel (50 & 250 ml.); dropper; test tube; heat; running water
Methol α-chloro-acrylate	Potassium permanganate	APMC	Yes	13.5 mm.	Comparator	Special jet bubblers; flowmeter; suction

Determination of	Using	Disc No.	Screen	Cells	Instrument	Equipment required
Nicotine	Silicomolybdic acid	NOF	—	50 ml.	Nessleriser	Pipettes (0.5, 1, 2, ml.); Gooch crucible (00); Whatman 42 filter discs; measuring cylinder (25 ml.); beaker (50 ml.); filter flask; suction; glass rod; volumetric flask (50 ml.); water bath; heat; thermometer
Nicotinic acid	Phosphomolybdic acid	5/33	—	13.5 mm.	Comparator	Pipettes (0—1 ml. graduated, 1, 2, ml.); measuring cylinder (25 ml.); heat; beaker (30 ml.); sintered crucible (G4); filter flask; suction; volumetric flask (50 ml.); water bath; thermometer
Nonox C.I.	Hydrogen peroxide	3/46	—	4 cm.	Special-Purposes Comparator	Balance; condenser; stirrer; flasks (50 ml. with stopper, 100 ml. graduated); funnel; filter-paper; heat; running water; pipettes (2, 10, 20 ml.); test-tube (6″ × 1″)
		MOW	—	50 ml.	Nessleriser	Balance; condenser; stirrer; flasks (50 ml. with stopper, 100 ml. graduated); funnel; filter-paper; heat; running water; pipettes (5, 10, 50 ml.)
Pentachlorophenates	Methylene blue	BT400	Yes	Acid wash tubes	Comparator	Pipettes (1, 5 ml.); rubber bulbs to fit pipettes
Pentachlorphenol	Copper-pyridine reagent	3/37A	—	5 mm.	Comparator	As for lauryl pentachlorphenol
Phenols — Monohydric	4-amino-antipyrine	3/43	—	13.5 mm.	Comparator	Measuring cylinder (50 ml.); volumetric flask (100 ml.); *p*H test papers; pipette (1 ml.)
Phenols — Total	Sulphanilic acid	NP	—	50 ml.	Nessleriser	Measuring cylinder (250 ml.); separating funnel (500 ml.); pipettes (5, 10 ml.); litmus paper
Pyridine	Copper silicomolybdic acid	NOG	—	50 ml.	Nessleriser	Pipettes (0.5, 1, 2 ml.); beaker (30 ml.); Gooch crucible (00); Whatman No. 42 paper discs; filter flask; suction; volumetric flask (50 ml.); water bath; heat; thermometer
Sugar	α-naphthol	3/29	—	13.5 mm.	Comparator	Pipettes (2.5, 5 ml.); glass-stoppered test tube
Thiophen	Alloxan	3/19	—	13.5 mm.	Comparator	Pipettes (2, 5 ml.); glass-stoppered test tube; water bath; heat; thermometer
Vitamin A	Antimony trichloride	—	—	1 cm.	Tintometer	Balance; burette (1 ml.); pipettes (2 ml.); volumetric flask (10 ml.)

The Determination of Amines

using Bromocresol Green

Introduction

This test[1] (Note 1) has been developed primarily for the determination of traces of long-chain aliphatic amines in water. Film-forming amines of this type have been used in water treatment for the prevention of growth of algae and also for the protection of steam lines. When used in this way, the determination of residual amine in cooling waters and condensates is essential for control purposes. Alternative methods[2–11] which have been proposed for this determination are unsuitable for field use, as they require exacting conditions for the production of accurate results. The present method, on the other hand, is capable of producing accurate results under field conditions, even in the hands of relatively unskilled operators.

Sulphonphthaleins react with primary, secondary, tertiary and quaternary amines and this test, although primarily developed for long-chain amines can be applied to the determination of other amines provided that the disc is recalibrated in terms of the particular amine being estimated.

Principle of the method

Amines react with sulphonphthaleins, in acid solution, to form a yellow complex. This complex is extracted from the aqueous solution by shaking with chloroform in a special comparator extraction tube. The intensity of the yellow colour in the lower (chloroform) layer after extraction is proportional to the concentration of amine, and is estimated by comparison with Lovibond permanent glass standards.

Reagents required

1. Amine test indicator tablets (equivalent to 0.5 ml. of a 0.1% solution of tetra bromo-*m*-cresol sulphonphthalein i.e. "bromocresol green," with an acid content equivalent to 15 mg. alkali, expressed as $CaCO_3$).
2. Chloroform, analytical reagent grade

The Standard Comparator Disc 3/58

This disc covers the range 1—10 p.p.m. of pure octadecylamine, and must be used in conjunction with the dulling screen supplied, with the disc, and in a Special Purposes Lovibond Comparator.

Technique

Take two of the special calibrated extraction tubes and carefully add chloroform to each, up to the 5 ml. mark. Place an indicator tablet in each tube. Into one tube introduce 10 ml. of sample, i.e. up to the 15 ml. mark. To the other tube add 10 ml. of distilled, or untreated, water to form the blank. Stopper both tubes and shake for 30 seconds.

Allow the chloroform layers to separate. If amine is present in the sample the lower (chloroform) layer will be coloured yellow. Place the sample tube in the right-hand compartment of the Special Purposes Lovibond Comparator and the blank tube in the left-hand compartment. The tubes have been designed so that the lower layers will cover the comparator apertures obviating the need to transfer the chloroform layers to separate comparator cells. Compare the colour of the sample with the Lovibond permanent glass standards in the disc, using north daylight wherever possible, and making sure that the dulling screen is in position in the comparator (Note 2).

Notes

1. This method, which is the subject of a patent application, has been developed by Houseman and Thompson Ltd. Permission to quote from their published literature is gratefully acknowledged.

2. If the intensity of the colour in the sample tube is greater than that of the 10 p.p.m. standard, the test should be repeated using a smaller volume of sample and making the total volume of water added up to 10 ml. by means of distilled water. The final reading should then be multiplied by the appropriate dilution factor, i.e. by 10 divided by the volume of sample used.

3. If the solution is insufficiently acidified, free ammonia tends to give a false reading. In the 10 ml. sample, there is sufficient acid in the tablet to allow up to 1,500 p.p.m. total alkalinity to be tolerated before the yellow colour is interfered with. Ammonium, iron and coppper salts and hydrazine do not interfere.

4. The apparatus supplied by Tintometer Ltd. for this test consists of :—

   Special Purposes Lovibond Comparator

   2 calibrated extraction tubes

   Disc 3/58 and screen

   Bottle for chloroform

   Bottle of amine test tablets

A fitted case to hold the above and ancillary equipment is available.

References

1. A. S. Pearce, *Chem. & Ind.*, 1961, 825
2. A. Milun and F. Moyer, *Anal. Chem.*, 1956, **28,** 1204
3. E. Carkhuff and W. Boyd, *J. Am. Pharm. Ass. Sci.*, Ed., 1954, **43,** 240
4. P. J. Lloyd and A. D. Carr, *Analyst,* 1961, **86,** 335
5. K. B. Coates, *Corrosion Tech.*, 1960, **7,** 46
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Determination of Carbohydrates

using orcinol

Introduction

The carbohydrate colour reaction of Tollens[1] was modified by Tillmans and Philippi[2] and used for the estimation of carbohydrates in various foodstuffs. This method has been still further modified so that it may be used with the Lovibond Comparator.

Principle of the method

The determination of carbohydrates in proteins may be carried out, without the necessity for preliminary hydrolysis, by virtue of the yellow to orange-red colour which they develop with orcinol. Carbohydrate molecules containing amino groups do not give this reaction.[2] By removal of the other carbohydrates present the method may be used for the estimation of glucose.

Reagents required

Orcinol (5-methyl-resorcinol)	..	0.2 g.
Sulphuric acid 66% v/v	..	99.8 g.

The Standard Lovibond Comparator Disc 3/8

The disc is graduated from 0.005 to 0.075 mg. of carbohydrate (reckoned as glucose) in 10 ml. final volume. The actual carbohydrate content of the 1 ml. sample is therefore 5 times that given on the comparator disc.

Technique

The material to be tested is dissolved, or suspended, in water and to 1 ml. of the mixture 10 ml. of 66% sulphuric acid containing 0.2% of orcinol are added. This mixture is heated for 12 minutes in a boiling water-bath. In general the whole of a biological material will dissolve under these conditions and there is not much charring. The mixture is cooled and diluted to 50 ml. with water. When again cool and when the air bubbles have risen to the top, the colour is measured by transferring some of the solution to a standard test-tube and placing in the right-hand compartment of the comparator. The disc is then revolved until the colour of the solution is matched by one of the glass colour discs, and the reading taken from the indicator recess in the bottom right-hand corner of the comparator.

Notes

1. If smaller amounts of solution and acid are used, for example 0.2 ml. of solution and 2 ml. of acid diluted to 10 ml., care must be taken to avoid the absorption of steam from the water bath.

2. Maltose, lactose, fructose, xylose, arabinose, sucrose, starch and glycogen give the reaction, glucosamine does not. Lactic acid, tartaric acid and acetone give negative reactions.

3. Formaldehyde and benzaldehyde interfere.

4. For details of suitable extraction procedures for the application of this method to soya beans, beans and lentils, wheat, (glutenin, gliaden and water-soluble protein) rye and hen's eggs reference should be made to Snell[3].

References

1. B. Tollens, "*Les Hydrates de Carbone*", Dunot & Pinat, Paris, 1918

2. J. Tillmans and K. Philippi, *Biochem Z.*, 1929, **215**, 36

3. F. D. and C. T. Snell, "*Colorimetric Methods of Analysis, Vol. II, Organic and Biological*", Chapman and Hall, London, 1937

The Determination of Carbon Disulphide

using diethylamine and copper acetate

Introduction

This method has been developed for determining the carbon disulphide content of benzoles, and can be adapted to cover the range .00004% to approximately .01% w/w sulphur as carbon disulphide in pure benzene. This disc is specified in the S.T.P.T.C. Method, Serial L.B. 17—57 and in British Standard 135 : 1953.

The test may usually be applied to samples containing unsaturated compounds, provided that, if the sample is coloured, a " blank " is used in compensation. Caution is required because some unsaturated hydrocarbons produce a colour with the reagents when no carbon disulphide is present.

Principle of the method

The sample is mixed with diethylamine and copper acetate, and the colour produced is compared with the standard colours in the disc. This was prepared in conjunction with the National Benzole Association Research Department, by carrying out the test on benzene solutions containing known quantities of carbon disulphide, treated in the same way.

Reagents required

1. Pure diethylamine that has been redistilled within 14 days of the test and kept in a closely stoppered bottle
2. A solution containing 0.03% w/v pure recrystallized copper acetate in absolute alcohol
3. Carbon disulphide-free benzene

The Standard Lovibond Comparator Disc 3/18

The disc covers the range 20γ to 120γ (0.02 to 0.12 mg.) sulphur (S) present as carbon disulphide, and is designed for use with the Special-Purposes Comparator and a 40 mm. cell.

Technique

Place an appropriate volume of the sample in one of the graduated cylinders, add 1 ml. of reagent 1 and 1 ml. of reagent 2. Make up the final volume to the 25 ml. mark with carbon disulphide-free benzene, stopper and shake. Stand in a water bath at 20° C. $\pm$ 5° C. for 8 minutes and then transfer the solution to one of the 40 mm. cells and place in the right-hand compartment of the comparator. A blank of the untreated sample is placed in the other 40 mm. cell in the left-hand compartment of the comparator, and the colour matched against the disc by north daylight, 10 minutes after the reagents were mixed.

The figure shown represents micrograms of sulphur in the original volume of the sample taken.

Note Carbon disulphide-free benzene may be prepared by refluxing pure benzole for nitration* for one hour with one third of its volume of alcoholic potash. After washing three times with a similar volume of water the benzole must be refractionated through a 12 pear column, the fraction distilling at 80° C.—80.5° C. being collected. Twenty five millilitres of this benzene, mixed with 1 ml. of diethylamine and 1 ml. of a 0.03% w/v solution of copper acetate shall not be darker than a similar quantity of the benzene alone when compared in standard Nessler cylinders.

* N.B.A. Specification No. 2, 1938

References

1. T. A. Dick, *National Benzole Association Memorandum R.*465, 1946
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3. S.T.P.T.C., *"Standard Methods for Testing Tar and its Products"*, 1957
4. *British Standard*, 135: 1953

The Determination of Carbon Tetrachloride

using pyridine

(Fujiwara reaction)

Introduction

Carbon tetrachloride is widely used as a dry-cleaning agent, both commercially and in the home. Its vapour is known to be highly toxic and the following method was devised by Daroga and Pollard[1] for the estimation of the vapour in air, and also for its estimation in soils. It is a modification of the well known Fujiwara reaction[2].

Principle of the method

In concentrated sodium hydroxide, carbon tetrachloride reacts with pyridine to produce a red colour which is matched against Lovibond permanent glass standards.

Reagents required

Sodium hydroxide (20% solution)
Pyridine (pure colourless)

The Standard Lovibond Comparator Disc 3/44

The disc covers the range 0.05 to 0.45 mg. of carbon tetrachloride.

Technique

Measure 10 ml. of sodium hydroxide into a 50 ml. graduated flask. Add 20 ml. pyridine and the test solution (see Notes 2 and 3). Loosely cork the flask, to avoid evaporation of pyridine, and immerse in boiling water for 5 minutes, shaking constantly. Cool under running water for 2 minutes. Transfer 10 ml. of the supernatant coloured pyridine layer to a standard test-tube and place in the right-hand compartment of the comparator. Match against the permanent glass standards, using north daylight wherever possible.

Notes

1. The amount of pyridine used must be measured accurately, as the colour produced is a function of the pyridine concentration.
2. ***Determination in air***
   Air is drawn, at 1 litre per minute, through 50 ml. of acetone contained in a Schott's gas-washing bottle,[3] fitted with a sintered-glass disc. The absorbed carbon tetrachloride is estimated colorimetrically as above.
3. ***Determination in Soil***
   Extraction by means of a solvent is impracticable. The carbon tetrachloride is recovered by steam distillation of a suspension of soil in 100 ml. of water to which 1 ml. of pyridine has been added. 50 ml. of distillate are collected in receiver containing 10 ml. of acetone, and the distillate made up to 100 ml. with acetone. Carbon tetrachloride is determined on an aliquot of this solution.
4. This method is applicable to any substance containing an RC-halogen group. Any such substance other than carbon tetrachloride will therefore interfere.
5. It is advisable to use a Pyrex volumetric flask to avoid cracking during the heating and cooling.

References

1. R. P. Daroga and A. G. Pollard, *J.S.C.I.*, 1941, **60**, 218
2. K. Fujiwara, *Sitzungber. Abhandl. Naturforsch. Ges. Rostock*, 1914, **6**, 1
3. P. H. Prausnitz, *Ind. Eng. Chem. (Anal. Ed.)*, 1932, **4**, 432

The Determination of Carotene

Introduction

It is generally agreed that from the carotene content of a lucerne or grass meal an indication of its general quality may be obtained. It can be said, as a rule, that if the carotene content is high, then the protein and Vitamin C content are high and the fibre is low. There are, of course, exceptions to this rule.

There is a government regulation that certain poultry rations must contain a grass meal of a stated carotene content. Many meals are sold according to their carotene and protein contents.

Previously the Lovibond Tintometer has been largely used for the colorimetric part of the assay for carotene, but this disc offers a more rapid method, and, as it is calibrated directly in mg. carotene, it is simpler to use.

Principle of the method

The β-carotene is estimated by means of the yellow colour of its solution in light petroleum. Before the β-carotene can be estimated however it is necessary to

(a) separate β-carotene from the non-active carotenoids

(b) remove all forms of chlorophyll

(c) prevent any possibility of isomerisation taking place[3]

The present method has been found to fulfil the requirements stated above. The carotene is extracted by means of light petroleum. All interfering materials are removed by chromatography on a bone-meal column, and the carotene is estimated in the eluate.

Reagent required

The carotene to be estimated colorimetrically should be dissolved in light petroleum (b. pt. 80°—100° C.).

The Standard Lovibond Comparator Disc 3/23

The disc covers the range .02—0.1 mg. carotene/100 ml. of solution, and is designed to be used with the Special-Purposes Comparator and 25 mm. cells.

Technique

Boil from 1 to 2 g. of grass meal with 50 to 60 ml. of light petroleum, of b.p. 80° to 100°C., under reflux for 1 hour on a steam bath. Cool the flask and contents and filter the extract onto a 2-inches by 1-inch column of bone meal. Rinse the flask and residue with small quantities of light petroleum. Apply suction to the column and elute with light petroleum.

The volume of the solution of carotene obtained by the above method is measured. As this solution is generally too concentrated for direct reading in the comparator, it is advisable to make two or three dilutions in light petroleum and take the mean of the results.

When a solution has been prepared which falls within the colour range of the disc, this is poured into one of the 25 mm. cells, which is placed in the right-hand compartment of the comparator. A blank of distilled water, is placed in another cell in the left-hand compartment, and the colour is matched against the disc by a good north daylight.

The figure shown at the indicator window represents the mg. carotene per 100 ml. petroleum in the actual solution under test. A simple calculation then gives the carotene content of the meal in milligrams per kilogram.

References

1. Carotene Committee of the Crop Driers Association, *Analyst*, 1941, **66**, 334

2. W. A. G. Nelson, *Analyst*, 1947, **72**, 200

3. L. Zechmeister, *Chem. Revs.*, 1944, **34** (2), 267

The Determination of Chloroform

using pyridine

(Fujiwara reaction)

Introduction

The widespread use of chloroform in anaesthesia has resulted in the search for a method of determining this substance in air, in body fluids and in tissue. The methods so far devised fall into two groups involving either decomposition of the chloroform, followed by estimation of chlorine either gravimetrically or volumetrically, or the direct colorimetric determination of chlorine. In general the methods involving decomposition of the chloroform are laborious without being sensitive, and colorimetric methods are therefore preferred for the determination of trace amounts. Daroga and Pollard[1] investigated the available colorimetric reactions and concluded that none of the existing methods was sufficiently reliable for routine use. They have therefore developed the following modification of the Fujiwara reaction[2].

Principle of the method

Chlorinated hydrocarbons react with pyridine, in the presence of strong alkali, giving a red colour. By careful control of the experimental conditions this colour can be stabilised and compared with Lovibond permanent glass standards.

Reagents required

Sodium hydroxide (20% solution)
Pyridine (pure, colourless)

The Standard Lovibond Comparator Disc 3/45

This disc covers the range 0.05 to 0.45 mg. of chloroform.

Technique

Measure 10 ml. of sodium hydroxide into a 50 ml. graduated flask. Add exactly 20 ml. of pyridine by means of a burette, and then add the test solution (see notes 3, 4, and 5). Loosely cork the flask to avoid evaporation of pyridine, and immerse the flask in boiling water for 5 minutes, shaking continually. Cool under running water for 2 minutes. The coloured pyridine layer now separates on top of the alkali. Transfer 10 ml. of this supernatant liquid to a standard test-tube and place in the right-hand compartment of the comparator. Using north daylight compare the colour with the permanent glass standards.

Notes

1. It is advisable to use a Pyrex volumetric flask to avoid breakage from thermal shock during heating and cooling.
2. The amount of pyridine added must be accurately measured as the colour developed is a function of the pyridine concentration.
3. ***Determination in air.***
   Air is drawn, at 1 litre per minute, through a Schott[3] apparatus containing 50 ml. of 0.05N hydrochloric acid, and the chloroform estimated on an aliquot of the resulting solution. The method is sensitive to 1 p.p.m. of chloroform vapour.
4. ***Determination in body fluids*** can be carried out directly by the method given above.
5. ***Determination in tissue.***
   Store the tissue in an air-tight jar in a refrigerator. When ice-cold, weigh about 100 g. into a distillation flask, add 100 ml. of water and sufficient tartaric acid to ensure that the solution is acid. Steam distill and collect the distillate in a 250 ml. conical flask containing 10 ml. of ice-cold 0.05N hydrochloric acid, cooling the receiver in ice. Collect nearly 200 ml. of distillate, dilute to 250 ml., mix and determine the chloroform on a 5 ml. aliquot by the technique described above.

References

1. R. P. Daroga and A. G. Pollard, *J.S.C.I.*, 1941, **60**, 218
2. K. Fujiwara, *Sitzungber Abhandl. Naturforsch. Ges Rostock*, 1914, **6**, 1
3. P. H. Prausnitz, *Ind. Eng. Chem. (Anal. Ed.)*, 1932, **4**, 432

The Determination of Cyanide

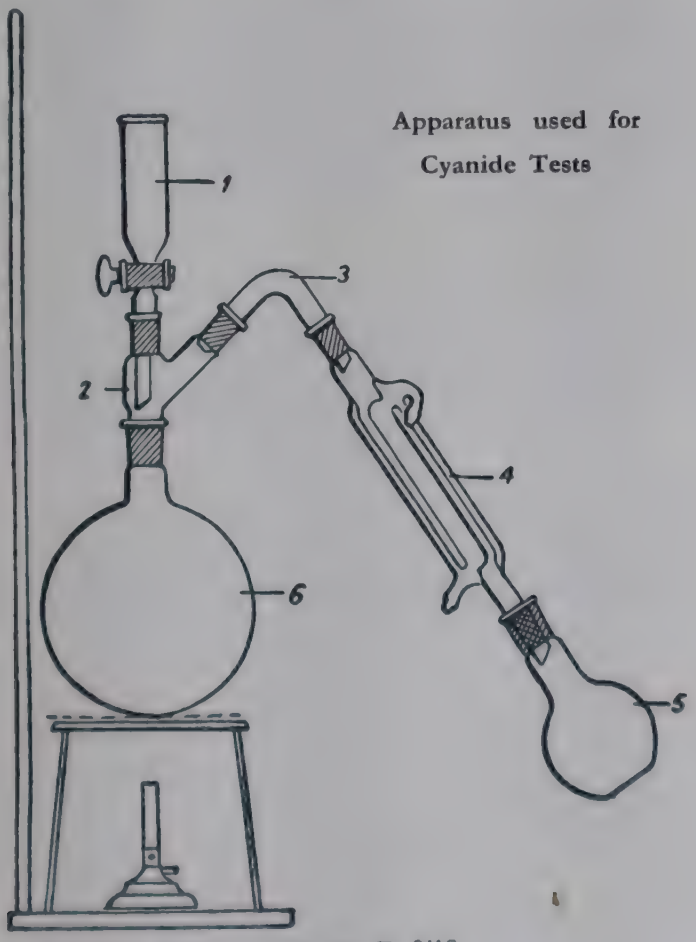
using pyridine-pyrazolone reagent

Introduction

The determination of traces of cyanide is of considerable general importance. Cyanides are one of the most toxic of all chemicals towards fish, and great care has to be taken to avoid discharging untreated cyanide wastes into rivers and fishing streams. Such toxic wastes may arise from electroplating and case-hardening processes and also from gas liquors. The destruction of cyanide in such wastes can be carried out by chlorination in alkaline solution. The cyanide content of effluents, after treatment, should not exceed 1-2 p.p.m. (as HCN). This test has been devised primarily for the estimation of cyanide in river waters, sewage and trade effluents.

Principle of the method

The method is based on those described by Epstein[1] and the American Public Health Association[2]. After treatment with chloramine-T cyanide reacts with a pyridine-pyrazolone reagent to give a pink colour which changes through mauve to blue on standing. The blue colour attained after 35 to 40 minutes is sufficiently stable to permit accurate visual comparison with Lovibond permanent glass standards. Chlorate, nitrate, phosphate, borate and sulphate do not interfere. Heavy metals, which might interfere, are removed by prior distillation of the sample.



Apparatus used for Cyanide Tests

1. Dropping funnel Cat. No. D. 3/12
2. Multiple adaptor Cat. No. M.A. 2/3
3. Bend Cat. No. S.H. 1/22
4. Double surface condenser. Cat. No. C. 5/12
5. 500 ml. Pyrex, flat bottom flask, medium neck B. 24
6. 1,000 ml. Pyrex, round bottom flask, short neck B.24

Also required :— B. 24 stopper B. 19 stopper

Catalogue numbers refer to :—
Quickfit & Quartz Ltd., Mill Street, Stone, Staffs.

Reagents required

1. Acetic acid—0.5 N. solution of glacial acetic acid (analytical reagent grade) in distilled water
2. Chloramine-T—0.2% aqueous solution. This solution must be prepared freshly each day.
3. Pyridine-pyrazolone reagent Dissolve 0.5 g. of 3-methyl-1-phenyl-5-pyrazolone in 250 ml. of a 50:50 mixture of ethyl alcohol (absolute, analytical reagent grade), and distilled water. This solution is stable. Dissolve, with shaking, 0.020 g. of bis-pyrazolone in 20 ml. of pyridine and make up to exactly 100 ml. with the 3-methyl-1-phenyl-5-pyrazolone solution. The resulting solution is unstable, even when stored in the dark, and must be prepared freshly each day.

The Standard B.D.H. Lovibond Nessleriser Disc NOC

This disc covers the range 0.02—0.2 p.p.m. of cyanide (CN^-).

Technique

Pipette 250 ml. of the sample into an all glass distillation apparatus (see figure), and distil to completion. Cool the distillate and make up to 250 ml. with distilled water, in a volumetric flask. Pipette 25 ml. of this distillate into a Nessleriser cylinder, and neutralise to *p*H 6 to 7, as shown by indicator papers, using the dilute acetic acid. Add 1 ml. of the chloramine-T solution, mix, and allow to stand for 2 minutes. Add 5 ml. of the pyridine-pyrazolone reagent, adjust the final volume to 50 ml. with distilled water and mix. Exactly 40 minutes after adding the chloramine-T reagent place the cylinder in the right-hand compartment of the Nessleriser, place an identical cylinder filled with distilled water in the left-hand compartment and match the colour of the sample solution against that of the standards in the disc, using north daylight whenever possible.

Note

It must be emphasized that the readings obtained by means of the BDH Lovibond Nessleriser and disc are only accurate provided that Nessleriser cylinders are used which conform to the specification employed when the discs were calibrated, namely, that the 50 ml. calibration mark shall fall at a height of 113 mm. $\pm$ 3 mm. measured internally.

References

1. J. Epstein, *Anal. Chem.*, 1947, **19**, 272
2. "*Standard methods for the Examination of Water, Sewage and Industrial Wastes*," American Public Health Association (A.P.H.A.) (10th edition), New York, 1955

The Determination of DDT

using nitric acid and alcoholic potash

Introduction.

This test developed by Martin and Batt[1] has two applications, firstly for the assessment of the performance of different types of spraying equipment, and secondly for the determination of levels of residues as a safeguard against possible hazards to consumers of crops treated with DDT. This latter aspect has been emphasized by the Working Party on Toxic Chemicals in Agriculture[2] which has drawn attention to the need for checking of such substances in food.

Principle of the method

The method depends on the separation of the DDT from the fruit, foliage or grain by carbon tetrachloride, nitration of the DDT, the recovery of the nitrated DDT by carbon tetrachloride and the development of a blue colour with alcoholic potash. The method determines the *p*, *p'*-isomer of DDT.

Reagents required

1. Carbon tetrachloride
2. Aluminium oxide, for chromatographic adsorption analysis
3. Fuming nitric acid—concentrated sulphuric acid, mixture of equal volumes
4. Potassium hydroxide 4N aqueous solution
5. Potassium hydroxide, 5% solution in absolute ethyl alcohol. The solution must be freshly prepared, colourless and should be filtered clear before use
6. Sodium sulphate anhydrous

Reagents of high analytical quality should be used.

The Standard Lovibond Comparator Disc 3/34

The disc covers the range of 0·025 to 0·35 mg. DDT. Owing to the brightness of the solution it is necessary to use a dulling screen in front of the sample to obtain a match.

A pair of 5 mm. comparator cells are required.

Technique

Extract a weighed quantity of the plant material with carbon tetrachloride. Suitable quantities for analysis are 5-10 grams of foliage, 25-50 grams of soft fruits or grain. Surface deposits may be removed by successive washing with warm carbon tetrachloride and decantation from a beaker. Alternatively, when more convenient, extraction may be made by continuous percolation with carbon tetrachloride.

Concentrate the extract to about 10 ml. volume by gentle distillation from a hot plate, cool, and pass through a column of 10 g. of alumina, previously wetted with 5 ml. of solvent, supported on cotton wool in a glass tube 150 mm. × 18 mm. diameter constricted at the lower end. Wash through with further 10, 10, 10, 10 and 5 ml. portions of carbon tetrachloride. Concentrate the percolate to about 5 ml. by gentle distillation and finally remove the solvent by passing a stream of dry air over its surface at 40°C.

To the residue add 2 ml. of nitric-sulphuric acid mixture, and heat in a boiling water bath for 10 minutes. Cool and slowly add 10 ml. of water. Cool again and neutralise the solution by the addition, with swirling and cooling, of 4N potash solution until litmus paper just turns blue.

Wash the solution with 15 ml. of carbon tetrachloride into a small pear-shaped separating funnel and extract by vigorous shaking for at least 30 seconds. Run the carbon tetrachloride layer into a second separating funnel and extract the aqueous layer with a further 5 ml. of carbon tetrachloride. Combine the carbon tetrachloride layers, wash with 3 ml. of water,

and run the carbon tetrachloride solution through anhydrous sodium sulphate, supported on cotton wool, into a 25 ml. graduated flask. Wash the separating funnel and sodium sulphate with a little more carbon tetrachloride and make up to 25 ml. volume.

Take a suitable aliquot part, concentrate to 0·5 ml. volume with a stream of dry air at 40°C., and add 5 ml. of alcoholic potash (see notes). Mix well. Pour into a 5 mm. comparator cell and place in the right-hand compartment of the Lovibond Comparator. In the left-hand compartment place a " blank " of absolute alcohol (or distilled water if the alcohol used is completely colourless) and make certain that the complementary screen is in position in the comparator in front of the sample solution. Exactly five minutes after the addition of the alcoholic potash solution hold the comparator facing a uniform source of white light—a north window whenever possible is best—and compare the colour produced in the test solution with the colours in the standard disc, rotating the latter until a colour match is obtained. The answer which is read from the indicator recess indicates mg. of DDT in the aliquot part taken of the final carbon tetrachloride solution of the nitrated DDT—e.g. if a fifth aliquot part was used and the original weight of plant material was 10 grams, the answer read from the disc must be multiplied by 5 to represent the amount of DDT in 10 grams of the material. This should be calculated to parts per million in the original plant material.

Notes

1. Apparatus with ground glass joints should be used throughout.

2. With most plant materials, the nitration mixture after the addition of water remains clear if less than about 0·05 mg. DDT is present. Quantities of DDT above 0·05 mg. are indicated by an opalescence, passing at higher levels to a precipitate. If little or no cloudiness develops, the whole of the carbon tetrachloride solution of the nitrated material should be taken for the colour development; if there is an appreciable cloudiness or precipitate, its extent should be used as a basis for deciding upon a suitable aliquot for the test.

3. Once the initial extraction has been made, the analysis should be completed with minimum delay.

4. The alcoholic potash should be added as soon as possible to the final solution of the tetranitro-DDT in 0·5 ml. of carbon tetrachloride. If crystals separate from the carbon tetrachloride solution the test should be rejected, since a low value may be obtained.

References

1. J. T. Martin and R. F. Batt, *Annual Report—Long Ashton Research Station*, 1953
2. *"Toxic Chemicals in Agriculture: Residues in Food"*, London, H.M.S.O., 1953
3. M. S. Schechter, S. B. Soloway, R. A. Hayes and H. L. Haller, *Anal. Chem.*, 1945, **17**, 704
4. E. T. Illing and W. H. Stephenson, *Analyst*, 1946, **71**, 310

The Determination of Anionic Synthetic Detergents (1)

using methylene blue

Introduction

The most common form of synthetic detergent is that classed as anionic, and the following test may be applied to determine how much is present in a solution. This test has been found useful in sewage laboratories, where trouble is experienced with "foaming," but may also prove useful in cases of suspected contamination of foods by cleaning materials.

Unfortunately there is no similar simple test for the non-ionic type of detergent.

Principle of the method

Anionic synthetic detergents combine with methylene blue to form a complex which is soluble in chloroform. By this means anionic detergents in very dilute solution can be extracted into a chloroform solution, and their concentration determined by comparing the colour intensity of the organic solution with permanent glass standards in the Lovibond Comparator.

Other substances found in sewage, such as naturally occurring compounds in urine, and some inorganic anions, also form a complex with methylene blue and are extracted with the anionic detergent. This difficulty can be overcome by extracting the detergent from two solutions of acidities corresponding to *p*H 3.25 and 0.7; whereas the detergent is extracted completely from both solutions the non-detergent is extracted in differing amounts. A combination of the results obtained by these two extractions enables the detergent concentration to be found to an order of accuracy adequate for routine checks on sewage.

Reagents required

1. Chloroform

2. Methylene Blue; B.P. quality, 0.175% solution in distilled water. Check quality as follows: Make a blank chloroform solution as described under "Technique" and examine 10 ml. in a comparator test tube. When viewed from above, looking down the tube against a white background, the solution should be only just perceptibly blue.

3. 2N sodium acetate

4. 10N sulphuric acid, made by adding 170 ml. of pure acid to 500 ml. water Determine the exact concentration of the solution by diluting a sample to N and titrating against N alkali.

Solutions which, when diluted, are of the correct methylene blue concentration and *p*H level are obtained by mixing the reagents as follows:—

Solution	*Solution A* (*p*H 3.25)	*Solution B* (*p*H 0.7)
Acetate	500 ml.	500 ml.
Methylene Blue	100 ml.	100 ml.
10N Sulphuric acid (quantities adjusted to provide this exact amount of 10N Sulphuric Acid)	95.2 ml.	323 ml.

Make each up to one litre with water.

The Standard Lovibond Comparator Disc 3/24

The disc covers the range from 1-16 p.p.m. active matter, measured as alkyl sulphates. For alkyl aryl sulphonates a factor must be used.

In order to distinguish between varying depths of the same methylene blue colour, a dulling screen is interposed in front of the solution. This has the effect of making the colours range from purple to blue-grey, and in consequence small variations in colour are readily distinguishable.

Technique

1. *Blanks*

Prepare blank solutions in the following way : Place 45 ml. of water in a 100 ml. separating funnel, and add 10 ml. of solution A and 10 ml. of chloroform. Shake vigorously for ½ minute. Allow to stand until the layers separate and run off the lower (chloroform) layer into a 50 ml. graduated flask through a small piece of cotton wool. Repeat the extractions another four times with 10 ml. portions of chloroform, and after the fifth extraction add chloroform to the flask until it contains 50 ml.

Make another blank solution, using solution B in place of solution A. A test tube full of one or other of these solutions is inserted in the left-hand hole behind the disc colours. The appropriate solution should always be in position when making measurements, and if evaporation occurs fresh chloroform must be added to make good the loss.

2. *Test*

Place in the separating funnel 10 ml. of sewage or effluent, and add 35 ml. of water. (If it is suspected that the detergent content may be low, as for example with final effluents, a larger volume can be taken ; but the combined volumes of sewage and water must amount to 45 ml. and the detergent value given by the comparator must be reduced proportionately). Add 10 ml. of solution A.

Extract the mixture with five 10 ml. portions of chloroform as described in section (1), and after the fifth extraction add chloroform until the flask contains 50 ml.

Place a portion of this solution in a comparator test tube and insert the tube into the right-hand hole in the comparator, behind the red screen. With a tube containing the "blank" from solution A in position, hold the comparator facing north daylight and rotate the disc until a match is obtained.

Note the reading shown in the aperture at the lower right-hand side of the comparator.

Repeat the above operations using the same volume of sewage or effluent as before, but substituting 10 ml. of solution B. Match the colour against a blank solution made from solution B and note the reading.

Interpretation of results

Let the reading obtained with the use of solution A be **a**
and „ „ „ „ „ „ „ „ B be **b**

Then the detergent concentration=(2b-a) parts of active matter (expressed as alkyl sulphates) per million parts of sewage. If it is desired to express the result in terms of anionic synthetic detergents other than alkyl sulphates, this value must be multiplied by a factor which must be obtained experimentally. For alkyl aryl sulphonates this factor is 1.2.

It will be understood that the commercially available detergents do not consist exclusively of active matter. The range of constitution for commercial products is normally 20—40% active matter, with the majority between 20% and 30%.

Notes

1. This disc was prepared using "Teepol," and the assistance of the Shell Refining and Marketing Company, Ltd., in preparing and checking the disc, is gratefully acknowledged.
2. A simplification of the above analytical procedure has been described by Degens *et al*[3].
3. A similar, but improved, method has also been suggested by Longwell and Maniece[4, 5].
4. The Standard Disc (3/24) may be used with both of these modified methods.

References

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2. H. C. Evans, *J. Soc. Chem. Ind.*, 1950, **69**, S76
3. P. N. Degens Junr., H. C. Evans, J. D. Kommer and P. A. Winsor, *J. App. Chem.*, 1953, **3**, 54
4. J. Longwell and W. D. Maniece, *Analyst*, 1955, **80**, 167
5. *cf* "Determination of Anionic Synthetic Detergents (2)" in "*Colorimetric Chemical Analytical Methods*", Tintometer Ltd., Salisbury, England, 1960

The Determination of Anionic Synthetic Detergents (2)

using methylene blue

Introduction

The test described in the preceding leaflet is based on "Teepol," an alkyl sulphate. Alkylbenzene-sulphonates are currently the most important anionic synthetic detergents in use and the present method has been developed to enable small amounts of these materials to be estimated. This test differs from previous tests in the method of extraction of the detergent. This has been the subject of several papers[1, 2, 3, 4, 5] and, of the various methods proposed, that of Longwell and Maniece[5] has been accepted as the 'official' test in Great Britain. The procedure is however still not completely satisfactory, particularly in comparisons with the 'referee' test[6] which uses infrared absorption spectroscopy. An alternative extraction procedure developed by Webster and Halliday[7] is also described. This is claimed to give much better agreement with results obtained using the 'referee' test.

The basis of calibration has been changed from "Teepol" to "Manoxol O.T.," as extensive investigation by Longwell and Maniece[5], showed that this material was a more stable and reproducible basis for standardisation.

Principle of the method

Alkylbenzenesulphonates combine with methylene blue to form a complex which is soluble in chloroform. The intensity of the colour in the chloroform layer is proportional to the amount of detergent present, which is estimated by comparison of the colour with that of the Lovibond permanent glass standards.

Other substances found in sewage and river waters also form a chloroform-soluble complex with methylene blue. In the preceding test this interference was overcome, at least in part, by a differential extraction at two different *pH's*. In the 'official' method of Longwell and Maniece[5] this extraction technique is replaced by an alkaline extraction followed by an acid wash. In the Webster and Halliday[7] method the sample is first subjected to acid hydrolysis, then to extraction with an amine solution and then finally to alkaline chloroform extraction and acid washing, as in the 'official' method.

Reagents required

1. Sulphuric acid (approximately 1.0N)
2. Sodium hydroxide solution (10% w/v, aqueous)
3. Chloroform (analytical reagent grade)
4. Light petroleum, boiling range 40° to 60°C.
5. 1-Methylheptylamine
6. *Neutral buffer solution* Dissolve 10g. of potassium dihydrogen orthophosphate in 800 ml. of distilled water, adjust to *pH* 7.5 with sodium hydroxide solution (Reagent 2) and dilute to 1 litre with distilled water.
7. *Alkaline buffer solution* Dissolve 10g. of disodium hydrogen orthophosphate in 800 ml. of distilled water, adjust to *pH* 10.5 with sodium hydroxide solution (Reagent 2) and dilute to 1 litre.
8. Methylene blue 0.2g. B.P. quality in 1000 ml. of distilled water. To reduce the blank value, extract sufficient of this solution for one day's use three times with chloroform, discarding the extracts (See Note 1).

The Standard Lovibond Comparator Disc 3/48

The disc covers the range 1 to 16 p.p.m. of active material, calculated as Manoxol O.T. (sodium dioctylsulphosuccinate) and based on a 10 ml. sample, and must be used with the dulling screen provided.

Disc 3/24, which is calibrated against 'Teepol' for use with the preceding test, may also be used with these tests provided that the figure obtained from the disc is multiplied by 1.4 to convert it to the equivalent value for Manoxol O.T.

Technique

(a) "Official" method[5].

Take sufficient sample to contain 20 to 150 μg. of anion active material (Note 2). Place sample in a separating funnel and make up to 100 ml. with distilled water*.

Add 10 ml. of the alkaline phosphate solution (Reagent 7), 5 ml. of methylene blue solution (Reagent 8) and 15 ml. of chloroform. Shake gently, twice a second, for 1 minute. Allow to separate. Break up any emulsion by gentle agitation with a polythene rod. Run the clear chloroform layer into a second separating funnel containing 110 ml. of distilled water, 5 ml. of methylene blue solution and 1 ml. of 1.0N sulphuric acid (Reagent 1). Rinse the first separating funnel with 2 ml. of chloroform and run this into the second separating funnel. Shake the second separating funnel and allow the layers to separate. Run the chloroform layer through a small funnel, plugged with glass wool which has been moistened with chloroform, into a 50 ml. volumetric flask. Rinse the second separating funnel with 2 ml. of chloroform and add this to the contents of the flask. Repeat the extraction of the contents of the two funnels twice more with further 10 ml. portions of chloroform, and combine the extracts in the volumetric flask. Make up to the 50 ml. mark with chloroform.

Thoroughly mix the contents of the volumetric flask by repeated inversion, and transfer an aliquot to a standard test tube. Place this tube in the right-hand compartment of the comparator. Repeat the above extraction procedure on reagents alone and place an aliquot of the resulting chloroform solution in an identical test tube in the left-hand compartment of the comparator. Make certain that the dulling screen is in position and compare the colour of the test solution with that of the permanent glass standards in the disc, using north daylight wherever possible.

(b) Webster and Halliday modification[7].

Place a volume of the sample containing 25 to 150 μg. of anionic detergent in a 400 ml. beaker, and adjust the volume to 100 ml. by dilution or evaporation as necessary. Use 100 ml. of distilled water as a blank and treat this in exactly the same manner as the sample.

Add 50 ml. of 1.0N sulphuric acid (Reagent 1), cover with a clock-glass and boil for 1 hour, adjusting the rate of boiling so that the volume is about 50 ml. at the end of this time. Add water if necessary to prevent the volume falling below this level. Neutralise the solution to phenolphthalein with sodium hydroxide solution (Reagent 2), taking care not to overshoot the endpoint, and transfer to a 250 ml. separating funnel with sufficient water to make a total volume of 100 ml. Use this same beaker for the subsequent collection of chloroform extracts.

Add 10 ml. of the neutral buffer solution (Reagent 6), and extract four times with separate 25 ml. portions of chloroform, to each of which has been added 1 drop of 1-methylheptylamine from a pipette dropper. (For samples that form persistent emulsions, increase the number of extractions to five; most emulsions can be readily broken by rubbing with a paddle-shaped polythene rod). For each extraction shake gently and evenly twice a second for two minutes. Combine the chloroform extracts in the 400 ml. beaker, add 5 ml. of water, and evaporate the chloroform and excess of amine on a steam or hot-water bath. Discard the aqueous layer from the separating funnel, which can be used, without washing, for the extraction with light petroleum.

Wash the sides of the beaker with 10 to 15 ml. of water, warm for a few minutes on the bath to ensure complete dissolution, and transfer to the separating funnel with sufficient water to make a total volume of 50 ml. Reserve the beaker for the subsequent collection of light

petroleum extracts. Add 10 ml. of neutral buffer solution, and extract four times with 25 ml. portions of light petroleum, to each of which has been added 1 drop of 1-methylheptylamine. Shake for 2 minutes for each extraction. Run the aqueous layer, as necessary, into a second separating funnel, and combine the light petroleum extracts in the 400 ml. beaker.

Discard the aqueous layer after the fourth extraction, place 15 ml. of methanol containing 1 drop of 1-methylheptylamine in the separating funnel, and shake for 30 seconds. Run the methanol into the second separating funnel, and again shake for 30 seconds. Run the methanol into the beaker containing the light petroleum extracts. Repeat the rinsing procedure with 25 ml. of water, and add this to the methanol-light petroleum mixture.

Evaporate the light petroleum on the steam or hot-water bath, and then boil on a hot-plate until free from excess of amine, as indicated by odour and by the solution becoming colourless to phenolphthalein. Maintain the volume at approximately 50 ml. by adding water as necessary. Add 5 ml. of sodium hydroxide solution (Reagent 2), and boil gently for 15 minutes. Occasionally rinse the sides of the beaker with water to maintain the volume at approximately 50 ml.

Remove all traces of 1-methylheptylamine from the separating funnel by rinsing with alcohol-hydrochloric acid mixture and then with water, and discard the rinsings. Transfer the sample solution to the separating funnel, neutralise to phenolphthalein with dilute sulphuric acid (Reagent 1) and add 1 drop of dilute sodium hydroxide solution (Reagent 2 diluted 1-250, i.e. 1 drop in 10 ml. of distilled water) to adjust the *pH* to between 8 and 10.

Cool and proceed as from * in the 'official' method.

Notes

1. The extracted methylene blue solution should not be stored for more than 1 day, as any traces of chloroform retained may slowly decompose and eventually lead to high blanks.

2. It is generally impracticable to take more than 10 ml. of raw or settled sewage, owing to the degree of emulsion formation on shaking with chloroform, but it is possible to take up to 100 ml. of good quality effluent, river or drinking water when the detergent content is very low. If a sample volume other than 10 ml. is taken the final result is calculated from the reading obtained from the disc by means of the following formula :—

$$\text{Detergent concentration as p.p.m. in Manoxol O.T.} = \frac{\text{Disc reading} \times 10}{\text{Sample volume in ml.}}$$

References

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The Determination of Dichlorophene

(Panacide; 5:5′-dichloro-2:2′-dihydroxy-diphenyl-methane; 2:2′ methylene bis [4-chlorophenol])

using 4-aminoantipyrine

Introduction

Phenolic fungicides are extensively used as mildew preventatives for fabrics destined to be used in tropical climates. One of the compounds most extensively used for this purpose is dichlorophene.

The method which has been most generally applied to the determination of dichlorophene in fabrics is that of Gottlieb and Marsh[1]. Ashton[2] has modified the oxidation stage of the original method, in order to improve the stability of the reagent blanks, and the present test is an adaptation of Ashton's method. This test has been developed at the request of the British Standards Institution Committee formed for the revision of B.S. 2087.

Principle of the method

This method is based on the observation by Emerson[3], that 4-aminoantipyrine condenses with phenols, in the presence of an alkaline oxidising agent, to form an antipyrine dye. The condensation of dichlorophene and 4-aminoantipyrine in the presence of alkaline potassium periodate yields a red colour, the intensity of which is proportional to the concentration of dichlorophene. The intensity of the colour is estimated by comparison with Lovibond permanent glass standards.

Reagents required

1. *Buffer solution pH* 9·68

Borax, analytical reagent grade	13.75 g.
Sodium hydroxide, analytical reagent grade	1.125 g. *
Distilled water	1,000 ml.

* Alternatively, use 28.2 ml. of $N/_1$ NaOH, and reduce the amount of water accordingly.

2. 1% *4-aminoantipyrine solution*
   1 g. of 4-aminoantipyrine in 100 ml. of reagent 1.

3. 0·25% *Potassium periodate solution*
   1.25 g. of analytical reagent grade potassium periodate dissolved in 500 ml. of reagent 1.

The Standard Lovibond Comparator Disc 3/57

This disc covers the range 0.4 to 2.0% of dichlorophene, based on a 1 g. sample, and is calibrated for use with a 5 mm. cell.

Technique

Extract a 1 g. sample of the proofed cloth by boiling with four successive 25 ml. portions of reagent 1. Allow each individual portion to boil for 7 minutes before decanting from the cloth. Combine the four extracts, cool, add a further 100 ml. of reagent 1 and make up to 200 ml. with distilled water. Filter a portion and, from the filtrate pipette 4 ml. into a 50 ml. volumetric flask. Add roughly 30 ml. of reagent 1; accurately pipette in 1.5 ml. of reagent 2 ***followed by*** 10 ml. of reagent 3 (Note 2).

Dilute the solution to the 50 ml. mark with reagent 1 and mix well. Transfer a portion of the diluted solution to a 5 mm. cell and place in the right-hand compartment of the comparator.

Fill an identical 5 mm. cell with a blank solution, prepared by mixing 38.5 ml. of reagent 1, 1.5 ml. of reagent 2 and 10 ml. of reagent 3, ***mixed in that order*** (Note 2), and place the cell in the left-hand compartment. Five minutes after placing the solutions in the comparator match the colour of the test solution against the Lovibond permanent glass standards in the disc, using north daylight wherever possible.

Notes

1. This modification of Ashton's[2] method has been developed by the Laboratory Chemicals Division of British Drug Houses Ltd. Permission to quote this method is gratefully acknowledged.

2. The amino-antipyrine solution (reagent 2) must always be added, and well mixed before the periodate solution (reagent 3) is added.

It is also important to ensure that the temperature of this reaction remains within the range 17–20°C. if accurate results are to be obtained.

References

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3. E. I. Emerson, *J. Org. Chem.*, 1943, **8**, 417

The Determination of Formaldehyde

using chromotropic acid

(1:8-dihydroxynaphthalene-3:6-disulphonic acid)

Introduction

Formaldehyde is one of the most important industrial chemicals, being used in the manufacture of synthetic resins and lacquers; as an intermediate in the manufacture of dyestuffs, explosives and other chemicals; and in dyeing, bleaching, calico printing, etc. Its ability to render glue and gelatin almost completely insoluble has resulted in its use in the textile and printing industries as a waterproofing agent for fabrics, for fixing glues and sizes and for increasing the fastness of dyes to washing.

The antiseptic properties of formaldehyde are used in such pharmaceutical preparations as medicines, deodorants and general and internal antiseptics. It has been prohibited for use as a food preservative but may occasionally be found in both milk and meat. It is extensively used in the preservation of histological and anatomical specimens.

Principle of the method

When warmed with chromotropic acid, in concentrated sulphuric acid solution, formaldehyde gives a violet-red colour. The exact chemistry of this reaction is, as yet, unknown. It has been suggested[1] that the first step is probably a condensation of formaldehyde with an aromatic hydroxyl group of the chromotropic acid producing a hydroxydiphenylmethane derivative. This would be soluble in the sulphuric acid and, on contact with air, is assumed to lead to a coloured oxidation product. Alternatively it is suggested[2] that the action of the concentrated sulphuric acid causes a phenol-aldehyde condensation followed by oxidation to a quinoid compound.

The chromotropic acid solution does not react with acetaldehyde, proprionaldehyde, n- and iso-butyraldehydes, iso-valeraldehyde, cenanthol, crotonaldehyde, chloralhydrate, glyoxal or aromatic aldehydes. A yellow colour is produced with glyceraldehyde, furfural, arabinose, fructose and sucrose. If, however, the blank solution is made up with an equal amount of any of these materials, then 0.5 micrograms of formaldehyde may be detected in the presence of 100 times the quantity of sucrose or fructose and of more than 300 times the quantity of the other compounds. Other sugars, acetone and carboxylic acids do not react with the chromotropic acid reagent, neither do aromatic acids[2]. The halogen derivatives of aromatic acids, and also phenoxy-acetic acid, do react to produce a wine colour[3,4].

Certain organic compounds split off formaldehyde when treated with acids and therefore develop a colour with the acid solution of chromotropic acid; hexamethylene-tetramine, formaldoxime, cellulose formals etc.[2,5] are typical of such compounds.

This method may be adapted for other estimations. Formic acid may be determined in small quantities by reduction to formaldehyde by nascent hydrogen[1,2]. Glucose interferes with this determination because it is partially reduced to formic acid and thence to formaldehyde during the reduction. Similarly methyl alcohol may be estimated in the presence of ethyl and higher alcohols, sugars and other aldehydes, etc., if the oxidation of methyl alcohol to formaldehyde, with potassium permanganate, is carried out under specific conditions[2,6].

Some metals, such as titanium, hexavalent chromium, and iron, will interfere with this determination. Oxidising agents, such as chlorates and nitrates, also interfere[7].

Reagents required

1. Chromotropic acid sodium salt A special reagent prepared by B.D.H. Ltd., for formaldehyde determinations, and should be specified as such when ordering.
2. Sulphuric acid, concentrated (analytical reagent grade) The reagent solution is prepared by adding 98 ml. of sulphuric acid to 2 ml. of a 5% aqueous solution of the chromotropic acid salt. This solution must be prepared freshly each day. Great care must be exercised when adding the concentrated sulphuric acid to the aqueous solution, especially in the intitial stages of the addition.

The Standard B.D.H. Lovibond Nessleriser Disc NOD

The disc covers the range from 1—10 micrograms of formaldehyde in nine steps.

Technique

Take 4 ml. of the sample, or adjust the amount taken to exactly 4 ml., with distilled water, add 4 ml. of the chromotropic acid reagent rapidly, but with caution, mix, and allow to stand for 30 minutes. The heat developed accelerates the reaction and full colour development. Transfer to a standard Nessleriser glass and make up to 50 ml. with distilled water. Place the glass in the right-hand compartment of the Nessleriser. Fill an identical glass to the mark with distilled water and place in the left-hand compartment. Match the colour of the sample against the permanent glass standards in the disc using north daylight whenever possible. The reading on the disc gives the number of micrograms of formaldehyde in the amount of sample used.

Notes

1. Great care must always be taken in handling concentrated acids, and it must be remembered that the chromotropic acid reagent is still virtually concentrated acid.

2. It must be emphasized that the readings obtained by means of the BDH Lovibond Nessleriser and disc are only accurate provided that the Nessleriser glasses used conform to the specification employed when the disc was calibrated, namely that the 50 ml. calibration mark falls at a height of 113 ± 3 mm. measured internally.

References

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The Determination of Furfuraldehyde

using aniline acetate

Introduction

This test has been developed primarily for the determination of furfuraldehyde in whisky, as this gives useful evidence of the character of a whisky.

It has been shown that the test is of wider application, however, and a modification has been recommended by Lampitt, Hughes and Trace[2], for the estimation of furfuraldehyde in vinegar. Garratt[3] has suggested its application to the detection, and approximate estimation, of Japanese mint oil in peppermint oils. Garratt[4] has also shown that the test may be applied to the detection of light camphor oil (9.2 Lovibond red) in rosemary oil (0.8 red) or clove oil (23.0 red) in bay (1.4 red) and in pimento berry oil (1.1 red).

Principle of the method

The test is based on the red colour produced when furfuraldehyde reacts with aniline salts. Methyl-furfuraldehyde, hydroxymethyl-furfuraldehyde and formaldehyde do not produce the red colour.

Reagent required

Aniline acetate, prepared by mixing equal volumes of

Aniline, freshly redistilled
Glacial acetic acid
Distilled water

This **must be** freshly prepared, using analytical reagent grade chemicals.

The Standard Lovibond Comparator Disc 3/30

The disc contains colour standards for

0.25, 0.5, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3

grams furfuraldehyde per 100,000 ml. absolute alcohol.

The dulling screen supplied with the disc must always be used.

Technique

Distil 250 ml. of the whisky to be tested, very gently to avoid charring. When the contents of the distillation flask are down to about 20 ml., add about 10 ml. distilled water, and continue the distillation until about 240 ml. are collected. Make volume up to 250 ml. with distilled water. If the whisky is 30 under proof, then take about 275 ml. and distil 250 ml. as above, to ensure that the distillate for analysis is always about 43.4% by volume absolute alcohol.

To 10 ml. of the distillate add 0.5 ml. of the reagent, mix well, and shield from strong light for 15 minutes. The temperature of the reaction should be controlled to about 15°–20°C.

Place a " blank " of distilled water in a 13.5 mm. cell in the left-hand compartment of the comparator, and the test solution in a similar cell in the right-hand compartment, and match against the disc after **exactly** 15 minutes, using diffused north daylight. The screen must always be in place, to act as a complementary filter in front of the solution. The answers read from the indicator recess represent grams per 100,000 ml. of absolute alcohol.

References

1. Simmonds, "*Alcohol*," MacMillan, London, 1919
2. L. H. Lampitt, E. B. Hughes and L. H. Trace, *Analyst*, 1927, **52**, 260
3. D. C. Garratt, *Analyst*, 1935, **60**, 369
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The Determination of Hydrazine

using *p*-dimethylaminobenzaldehyde

Introduction

The use of hydrazine for the treatment of boiler feed water has been recommended by a number of workers[1, 2, 3]. Its main advantage in this application is claimed[1] to be its superior performance, as compared with sodium sulphite, in the removal of dissolved oxygen.

Several methods have been suggested for the determination of hydrazine, including oxidation with permanganate[4], potentiometric titration with iodine chloride[5] and colorimetric estimation using 2-nitro-1,3-indandione[6]. The present method is based on an A.S.T.M. test[7] and has been especially developed for the determination of the low concentrations of hydrazine encountered in the examination of boiler feed waters.

Principle of the method

p-Dimethylaminobenzaldehyde reacts specifically with hydrazine giving a yellow colour. The intensity of this colour, which is proportional to the concentration of hydrazine, is estimated by comparison with Lovibond permanent glass standards.

Reagents required

1. *p*-Dimethylaminobenzaldehyde reagent

p-dimethylaminobenzaldehyde (See Note 1)	4.0g.
Alcohol (absolute ethanol or absolute industrial methylated spirit, 74 O.P.)	200 ml.
Hydrochloric acid (36%)	20 ml.

2. Hydrochloric acid, 5N

The Standard B.D.H. Lovibond Nessleriser Disc NOH

The disc covers the range 0 to 10 microgrammes of hydrazine (N_2H_4).

Technique

Place a 10 or 25 ml. fresh sample of the boiler feed water (Notes 2 and 3) in a 50 ml. Nessleriser cylinder (Note 4). Add 1 ml. of 5N hydrochloric acid and 10 ml. of the *p*-dimethylaminobenzaldehyde reagent and mix thoroughly. Adjust the volume to 50 ml. with distilled water, mix and allow to stand for 10 minutes. Place the cylinder in the right-hand compartment of the Nessleriser. Fill an identical cylinder with 10 ml. of unreacted reagent and 40 ml. of distilled water, and place in the left-hand compartment. Match the colour of the sample with that of the permanent glass standards in the disc, using north daylight wherever possible.

Notes

1. It is essential to use a specially purified grade of *p*-dimethylaminobenzaldehyde for this determination, as the yellow colour of solutions prepared from ordinary grades of this material prevents correct readings being obtained. The colour of reagent 1, in a filled Nessleriser cylinder should not exceed that of the zero standard in the disc.
2. On a 10 ml. sample the range of the disc is from 0 to 1.0 part per million, and on a 25 ml. sample from 0 to 0.4 part per million of N_2H_4.
3. The sample must not be collected at a temperature exceeding 21°C. (70°F.), and an efficient cooling coil should be fitted at the sampling point, if necessary, to ensure that this temperature is not exceeded.
4. The readings obtained by means of the B.D.H. Lovibond Nessleriser and disc are only accurate provided that the Nessleriser glasses which are used conform to the specification employed when the disc was calibrated, that is that the 50 ml. calibration mark shall fall at a height of 113 ± 3 mm. measured internally.

References

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2. E. R. Woodward, *Petroleum Refiner*, 1956, **35**, 208
3. G. Genin, *Chaleur & ind.*, 1957, **38**, 57
4. M. Issa and R. M. Issa, *Anal. Chim. Acta*, 1956, **14**, 578
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6. G. Vanags and M. Mackanova, *Zhur. Anal. Khim.*, 1957, **12**, 149
7. A.S.T.M. Designation, D1385, 57T

The Determination of Lauryl Pentachlorphenol and Pentachlorphenol

using copper pyridine reagent

Introduction

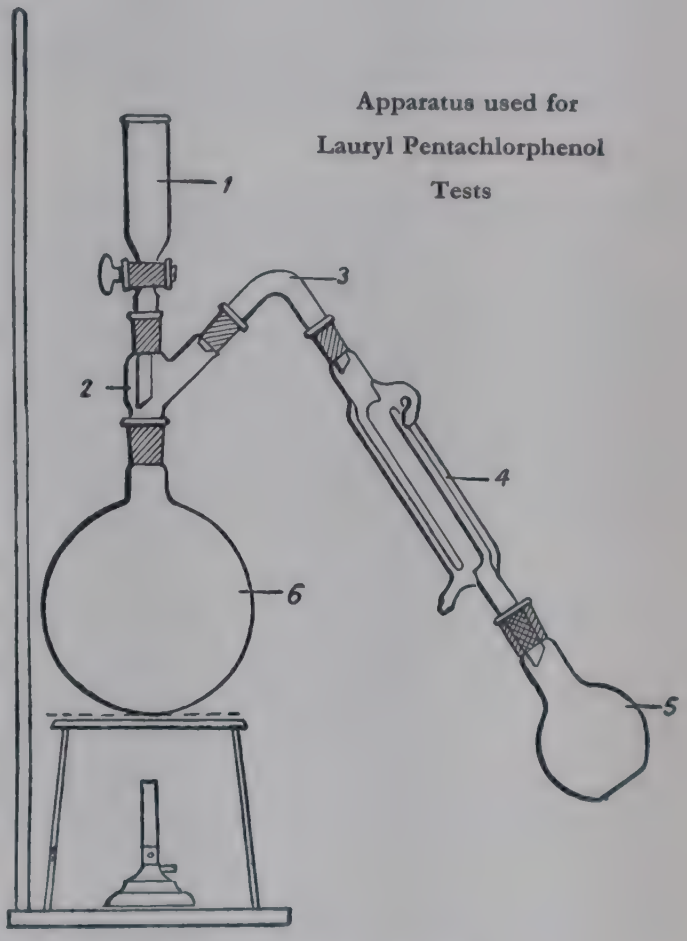
Lauryl pentachlorphenol and pentachlorphenol are used as rot-proofing agents and as fungicides on flax and jute textiles, fire hose and canvas. Certain official specifications (e.g. British Standard 2087:1954) lay down the minimum amount of the agent which will be acceptable or adequate for the purpose. In such cases, a chemical test is required to ascertain that these conditions have been fulfilled. The following test has been designed for this type of control.

Principle of the method

The lauryl pentachlorphenol is saponified to liberate pentachlorphenol and lauric acid as their sodium salts. The solution is then acidified in order to liberate the free phenol, which is separated from other organic debris by steam distillation and concentrated by extracting the distillate with chloroform.

The chloroform extract is treated with a copper-pyridine reagent, which forms a copper-pyridine-pentachlorphenol complex soluble in chloroform, and imparts an intense brown colour to the solution.

The intensity of this solution is matched against Lovibond permanent glass standards.



Apparatus used for Lauryl Pentachlorphenol Tests

1. Dropping funnel Cat. No. D. 3/12
2. Multiple adaptor Cat. No. M.A. 2/3
3. Bend Cat. No. S.H. 1/22
4. Double surface condenser Cat. No. C. 5/12
5. 250 ml. Pyrex, flat bottom flask, medium neck B. 24
6. 1,000 ml. Pyrex, round bottom flask, short neck B. 24

Also required:— B. 24 stopper
B. 19 stopper

Catalogue numbers refer to:—
Quickfit & Quartz Ltd., Mill Street, Stone, Staffs.

Reagents required

1. 4N sodium hydroxide solution (160 g. per litre)
2. Concentrated hydrochloric acid (35—36%) Sp. G. 1.18.
3. Chloroform, (analytical reagent grade)
4. Copper sulphate solution Dissolve 3 g. of copper sulphate (analytical reagent grade, $CuSO_4 5H_2O$) in 60 ml. of distilled water.
5. Pyridine, redistilled

The Standard Lovibond Comparator Discs, 3/37A and 3/37B

Disc A Pentachlorphenol 0.1% to 0.9%.

Disc B Lauryl Pentachlorphenol 0.2% to 1.0%.

These discs must be used in conjunction with a 5 mm. cell.

Apparatus

The apparatus used is as detailed in the accompanying diagram. During refluxing the double surface condenser can function as a reflux condenser.

It is preferable to use glassware with ground glass joints, such as is supplied by Quickfit & Quartz Limited, Stone, Staffordshire.

Technique

The determination of lauryl pentachlorphenol on all materials other than wool

Weigh 5 g. of material, cut up and place in a litre flask together with 100 ml. of 4N sodium hydroxide. A few pieces of unglazed porcelain are also introduced into the flask to prevent bumping. Boil the mass under reflux for two hours.

At the end of this period, fit up the flask for distillation. Add 250 ml. of distilled water and 80 ml. of concentrated hydrochloric acid to the flask through the dropping funnel and distill the mass until approximately 200 ml. of distillate is collected. The distillate is then extracted with 20 ml. of chloroform, the condenser being washed out by the chloroform on its way to extract the distillate.

Separate the chloroform layer in a 250 ml. separating funnel and transfer it to a 50 ml. separating funnel ; add 30 drops of copper sulphate solution and 30 drops of pyridine to this chloroform solution, shake well and allow to separate.

Filter the coloured chloroform solution into a test tube and from this fill a 5 mm. cell. Place in a Lovibond Comparator. Hold the comparator about 18 inches from the eye, facing a uniform north daylight, and compare the intensity of the colour with colours on the lauryl pentachlorphenol disc B. The percentage of true lauryl pentachlorphenol contained in the fabric is read off direct. (see Note 1).

The determination of lauryl pentachlorphenol on wool

Owing to the presence of sulphur compounds, produced during saponification when wool is present, trouble may be encountered when carrying out lauryl pentachlorphenol estimations if hydrochloric acid is used for acidification prior to distillation.

This may be obviated by using 100 ml. of 8N formic acid solution (containing zinc sulphate) in place of the 80 ml. of concentrated hydrochloric acid. Prepare this 8N formic acid solution as follows :—

Dissolve 100 g. of zinc sulphate ($ZnSO_2$, $7H_2O$) in 500 ml. of distilled water, add 433 g. 85% formic acid and make up to one litre with distilled water.

British Standards Institution method

B.S. 2087 : 1954 gives a standard method for determination of lauryl pentachlorphenol on textile materials, but it has been found that, when using this method on some fabrics, a pronounced reddening of the chloroform solution is obtained after the addition of the copper pyridine indicator due, presumably, to the action of the residual concentrated hydrochloric acid on the textile material.

The determination of pentachlorphenol on fabrics

The estimations are carried out substantially as directed for the estimation of lauryl pentachlorphenol. The only modification is that, as the pentachlorphenol is present as a protein complex in wool, it will only be necessary to reflux with sodium hydroxide for a short period in order to disintegrate the wool before acidification and distillation.

When estimating pentachlorphenol, **use Disc A,** as the colours are slightly different from those developed in the other test, and matching would be difficult. The percentage of true pentachlorphenol is read direct from this disc, as these colours were matched against actual solutions of pentachlorphenol.

Notes

1. The word "true" is used because these glass colour standards were matched against actual solutions of lauryl pentachlorphenol and not against secondary standards.

If the colour intensity obtained is darker than the darkest colour in the disc, it is only necessary to dilute the chloroform solution with more chloroform and then, after comparing the colour, multiply by the dilution factor the % lauryl pentachlorphenol figure obtained.

2. These discs were prepared in conjunction with the laboratories of Catomance Limited, Welwyn Garden City, and thanks are expressed to them for their skilled assistance in modifying the technique for these special tests. They will be pleased to answer any technical questions regarding the chemistry of the tests.

Reference

R. W. Moncrieff, " *Mothproofing,* " Leonard Hill Limited, London, 1952

The Determination of Methyl α-chloro-acrylate

using potassium permanganate

Introduction

The determination of methyl α-chloro-acrylate in the atmosphere is important in the control of working conditions in the manufacture of poly-methyl α-chloro-acrylate polymer.

Principle of the method

Methyl α-chloro-acrylate reacts with dilute aqueous solutions of potassium permanganate, producing a colour which ranges from red through orange-red to orange-yellow as the concentration of methyl α-chloro-acrylate increases.

Reagent required

0.001*N* potassium permanganate solution

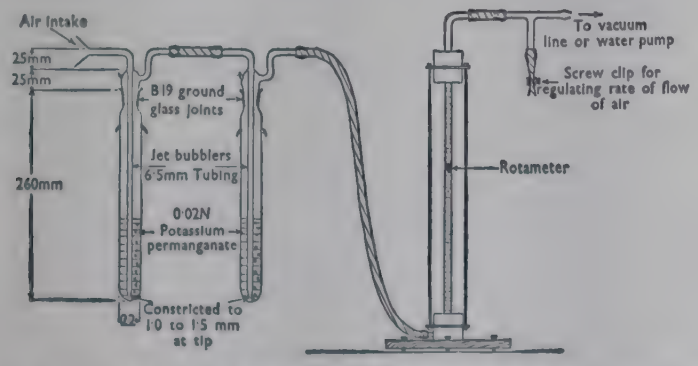
The Standard Lovibond Comparator Disc APMC

The disc covers the range 0–100γ (0—0.1 mg.) of methyl α-chloro-acrylate.
A dulling screen must be used with this disc.

Technique

The methyl α-chloro-acrylate is absorbed in 'jet' bubblers[1] containing 10 ml. of 0.001*N* potassium permanganate solution. Two of these bubblers are used in series, the purpose of the second being to trap any acrylate which slips through the first bubbler. The dimensions of these bubblers are :—

Length 26 cm., internal diameter 22 mm., tube diameter 6.5 mm. drawn out to 1–1.5 mm. at the tip.



Air is drawn through the bubblers at 1.5 litres a minute until the colour of the permanganate solution in the first bubbler appears to be suitable for colour comparison. The airflow is then discontinued and the duration of sampling is recorded.

The contents of the first bubbler are drained into a 13.5 mm. cell which is placed in the right-hand compartment of the comparator, and a similar cell, filled with distilled water, is placed in the left-hand compartment. The colour of the sample is then matched with the permanent glass standards in the disc, using a uniform source of white light—north daylight whenever possible. The reading in the indicator window gives the amount, in mg., of methyl α-chloro-acrylate contained in the solution.

The contents of the second bubbler are treated in the same manner and the amount of acrylate found is added to that from the first bubbler. The concentration of methyl α-chloro-acrylate in the air is calculated as follows :—

$$\text{Concentration (mg./m}^3\text{)} = \frac{\text{Total reading in mg.}}{\text{Sampling time in min.}} \times \frac{1000}{1.5}$$

This concentration is converted to parts per million v/v at 20°C and 760 mm. pressure by dividing by 5.

Notes

1. Other acrylates, such as methyl methacrylate interfere with this method, but are unlikely to be present in the same atmosphere, particularly in a plant manufacturing polymethyl α-chloro-acrylate.

2. This method was developed by the staff of I.C.I. (Plastics) Ltd.

Reference

1. J. Haslam, S. M. A. Whettem and W. W. Soppet, *Analyst*, 1951, **76**, 628

The Determination of Nicotine

using silicomolybdic acid

Introduction

Nicotine is widely used as an insecticide in horticulture, and the following method was developed by Sutherland, Daroga and Pollard[1] primarily for the determination of small amounts of nicotine in soils. The method is a modification of that proposed by Hofmann[2], and may also be used for the determination of nicotine in tobacco provided that the nicotine is removed from the non-volatile alkaloids and the proteins by steam distillation.

Principle of the method

Nicotine is precipitated as its complex with silicomolybdic acid. This precipitate is then reduced with glycine and sodium sulphite. The blue colour produced is compared with permanent glass standards, using either a Tintometer or a B.D.H. Lovibond Nessleriser.

Reagents required

1. Silicomolybdic acid

Molybdic anhydride	14.4 g.
Sodium hydroxide (Normal solution)	100 ml.
Silica (as sodium silicate solution)	0.7 g.
Hydrochloric acid (10% solution)	

The molybdic anhydride is dissolved in the sodium hydroxide by warming, and the sodium silicate solution added. Hydrochloric acid is then added slowly, with stirring, until the solution becomes green. The solution is diluted to 900 ml. and heated on a boiling water-bath for 3 hours. After cooling, the solution is stored at ambient temperature for 24 hours, the excess silica is filtered off, and the filtrate is diluted to 1 litre.

2. Wash liquid

10% of sodium chloride in 1% hydrochloric acid

3. Reducing solution

Glycine	1 g.
Sodium sulphide (20% aqueous soln.)	15 ml.
Ammonia solution (d 0.880)	5 ml.

Dissolve the glycine in the sodium sulphide solution, add the ammonia slowly, while stirring. Dilute the solution to 100 ml.

The Standard B.D.H. Lovibond Nessleriser Disc NOF

This disc covers the range 0.2 to 1.8 mg. of nicotine.

Technique

2 ml. of reagent 1 are added to the solution of nicotine in 15 ml. of 1% hydrochloric acid. The solution is warmed and shaken and is allowed to stand at ambient temperature for at least 2 hours. The precipitate is then collected on a disc of No. 42 Whatman paper in a small (No.00) Gooch crucible. The beaker and precipitate are washed with successive quantities of 1.0, 0.5 and 0.5 ml. of reagent 2. 2-3 ml. of reagent 3 are pipetted into the beaker and any residual precipitate is dissolved. The solution is poured into the Gooch crucible and the precipitate dissolved by stirring with a glass rod. The solution is then sucked into the filter flask and the beaker and filter washed through with further amounts of reagent 3. The combined filtrate and washings are poured into a 50 ml. graduated flask and diluted to the mark. The colour is then developed by heating the flask and contents on a water-bath at 45°C. for 25 minutes.

The coloured solution is transferred, after cooling, to a standard Nessleriser tube and placed in the right-hand compartment of a B.D.H.-Lovibond Nessleriser. The colour is compared with the colours in the standard disc using north daylight as the illuminant.

Notes

1. It is important that the instructions are followed exactly in all particulars as any variation will affect the final colour.
2. The Nessleriser tube used must be identical with the standard tube used in calibrating the disc, if accurate results are to be obtained, i.e. the calibration mark must fall at a height of 113 ± 3 mm. measured internally.

Reference

1. G. L. Sutherland, R. P. Daroga and A. G. Pollard, *J.S.C.I.*, 1939, **58,** 284
2. R. Hofmann, *Biochem, Z.*, 1933, **260,** 26

The Determination of Nicotinic Acid

using phosphomolybdic acid

Introduction

Nicotinic acid has been identified as one of the constituents of the Vitamin B complex. It is used in medicine for the treatment of deficiency diseases, such as pellagra, and of other nutritional disorders. The estimation of the nicotinic acid content of foodstuffs is therefore important, particularly in the case of some special dietary preparations. Its estimation in urine affords a method of control in cases where nicotinic acid is being administered for medicinal purposes. The present method was developed by Daroga[1] for use with the Tintometer, but has been adapted for use with the Lovibond Comparator.

Principle of the method

The nicotinic acid is precipitated with phosphomolybdic acid. After the removal of excess reagent the nicotinic acid-phosphomolybdate complex is reduced with stannous chloride to produce the molybdenum blue colour, which is compared with permanent glass standards in the Lovibond Comparator.

Reagents required

1. Phosphomolybdic acid 2.5 g.
   Hydrochloric acid (20%) 20 ml.
   Distilled water 20 ml.

   The phosphomolybdic acid is dissolved by warming in the hydrochloric acid and water. The solution is cooled, filtered, and the filtrate diluted to 50 ml.

   This reagent is stable for 4 weeks if stored in darkness.

2. Wash liquid
   Glacial acetic acid 5 ml.
   Distilled water 95 ml.

3. Sodium hydroxide (0.1 N approx.)

4. Reducing solution
   Stannous chloride 10 g.
   Hydrochloric acid (conc.) 25 ml.

   This reagent deteriorates on keeping and should be freshly prepared on the day it is to be used.

The Standard Lovibond Comparator Disc 5/33

This disc covers the range 0.4 to 1.2 mg. of nicotinic acid.

Technique

To 1 ml. of the sample solution (which should contain 0.1—1.0 mg. of nicotinic acid), in a 30 ml. beaker, is added 0.2—0.3 ml. of reagent 1. The solution is warmed until the precipitate is dissolved, a further 0.1 ml. of reagent 1 being added if required. The solution is cooled to room temperature and allowed to stand for at least 2 hours. The precipitate is collected, using a sintered glass crucible of porosity G4, and the crucible and beaker are washed with three successive portions of 1 ml. of reagent 2, the suction being shut off during each addition of wash liquid. After the final washing the crucible is sucked dry for 2 minutes, and the filter flask is then emptied and washed.

1-2 ml. of reagent 3 are pipetted into the crucible and allowed to stand for 2-3 minutes to dissolve the precipitate. The solution is sucked into the flask and the filter washed with 15 ml.

of water at 25°C. The liquid and washings from the filter flask are transferred to the beaker used for the precipitation, and 1 ml. of reagent 4 is added. The solution is then transferred to a 50 ml. graduated flask and diluted to the mark. The flask is maintained at 25°C. for 5 minutes, to develop the full colour. 10 minutes after the addition of reagent 4 a standard test-tube is filled to the mark with solution from the flask, placed in the right-hand compartment of the comparator, and the colour compared with the permanent glass standards immediately.

Notes

1. Maximum colour is obtained within 5 minutes at 25°C. and the colour commences to fade after 15 minutes. Matching of the colour with the standards should, therefore, always be carried out 10 minutes after the reducing agent (reagent 4) is added.

2. If the concentration of nicotinic acid falls outside the limits of the standard colours the concentration of the test solution should be adjusted by concentration, or dilution, as required. It has been demonstrated[1] that a solution of nicotinic acid in excess of dilute hydrochloric acid may be evaporated without loss.

3. Extraction of nicotinic acid from foodstuffs.

   To a suspension of the foodstuff in 250 ml. of distilled water add 3 g. of calcium oxide and 20 g. of sodium chloride. Pass a current of steam through the heated suspension and collect 400 ml. of distillate in 20 ml. of 20% hydrochloric acid. Evaporate the distillate to about 2 ml. and determine the nicotinic acid in an aliquot as described.

4. Extraction of nicotinic acid from urine.

   Two compounds known to occur in normal human urine, trigonelline and methylpyridinum hydroxide, are likely to be precipitated by reagent 1. These compounds must, therefore, be removed from the urine before releasing the nicotinic acid.

   Dilute 100 ml. of urine to 250 ml. with distilled water. Steam distil for 20 minutes and reject the distillate. Allow the residual mixture of urine and water to cool, add 3 g. of calcium oxide and 20 g. of sodium chloride and proceed as in Note 3.

Reference

1. R. P. Daroga, *J.S.C.I.*, 1941, **60**, 263

The Determination of Nonox C.I.

(N.N′ di-β-naphthyl *p*-phenylene diamine)

using hydrogen peroxide

Introduction

Polythene, when heated, is oxidised by atmospheric oxygen with consequent impairment of its physical properties. In order that moulding and extrusion processes may be carried out at elevated temperatures it is necessary to add an antioxidant to the polythene to prevent oxidation. Nonox C.I. is one of the materials most commonly used for this purpose. This test has been devised to enable the concentration of Nonox C.I. in the plastic to be controlled.

Principle of the method

The polythene is dissolved in hot toluene, under reflux conditions, and then reprecipitated, by the addition of ethyl alcohol, leaving the Nonox C.I. in solution. After filtration the solution is treated with acid hydrogen peroxide, and the green colour produced is compared with permanent glass standards.

Reagents required

1. Toluene (B.D.H. Laboratory Reagent—sulphur free)
2. Ethyl Alcohol—95%
3. Hydrogen peroxide reagent

Sulphuric acid (20% v/v)	25 ml.
Hydrogen peroxide (100 vols., analytical reagent grade)	4 ml.
Distilled water	to 100 ml.

Standard Lovibond Discs, Comparator Disc 3/46 and Nessleriser Disc MOW

Disc 3/46 covers the range 0.10% to 0.26% of Nonox C.I. in steps of 0.02% and is designed for use with the Special Purposes Comparator and 4 cm. cells. Disc MOW, covers the range 0.005% to 0.045% of Nonox C.I. in steps of 0.005%.

Technique

Weigh the appropriate amount (Comparator test 0.400 g ; Nessleriser test 1.00 g.) of the polythene sample into a clean 50 ml. round bottomed "Quickfit" flask. Pipette 10 ml. toluene into the flask, place on a heating mantle and fit to the neck of the flask a short condenser through the middle of which runs an air, or electrically, driven stirrer. Start the stirrer and heat the contents of the flask until all the polymer has dissolved. This usually takes 15-30 minutes. Turn off the stirrer and wash down the condenser with 15-20 ml. ethyl alcohol. Remove the flask from the condenser, stopper with a glass stopper and shake vigorously to precipitate the polymer. Cool and filter through a Whatman No. 31 filter paper, into a 100 ml. flask. Wash the flask and the precipitate thoroughly with ethyl alcohol and make up the total volume of the filtrate to 100 ml.

(a) ***Comparator test***

Pipette 20 ml. of the filtrate in to a 6″ × 1″ test tube. Add 2.0 ml. of the hydrogen peroxide reagent, mix well and allow to stand. After 15 minutes pour the solution into a 4 cm. glass cell and place in the right-hand compartment of a Speical-Purposes Comparator. Place an equal volume of distilled water in an identical cell in the left-hand compartment and compare the green colour of the sample solution with that of the standards in the disc, using north daylight whenever possible. Make further comparisons at 5 minute intervals until the maximum colour development is attained (this may take up to 45 minutes). The reading on the disc at maximum colour development gives directly the percentage of Nonox C.I. in the sample of polythene.

(b) ***Nessleriser test***

Transfer 50 ml. of the sample solution to a standard Nessler cylinder. Add 5 ml. of the hydrogen peroxide reagent, mix well and set aside for 5 minutes. Place the Nessler cylinder in the right-hand compartment of the Nessleriser and place an identical cylinder filled with distilled water in the left-hand compartment. Compare the green colour of the sample solution with the colours of the standards in the disc, using north daylight whenever possible. Make further comparisons at 5 minute intervals until maximum colour development is attained (this may take up to 40 minutes). The reading on the disc at maximum colour development gives directly the percentage of Nonox C.I. in the sample of polythene.

Notes

1. This test was developed by Dr. J. Haslam & Mr. D. C. M. Squirrell in the laboratories of Plastics Division, Imperial Chemical Industries, Ltd.
2. It is essential that the exact amounts of polythene specified for the tests are used, if the disc readings are to give a direct percentage of Nonox C.I.
3. It must be emphasised that the readings obtained by means of the B.D.H. Lovibond Nessleriser and disc are only accurate provided that Nessler cylinders are used which conform to the specification employed when the discs were calibrated, namely that the 50 ml. mark falls at a height of 113 mm. ± 3 mm. measured internally.

The Determination of Pentachlorophenates

using methylene blue

Introduction

Pentachlorophenates are widely used for mildew proofing and for algae control in waters. They are also used as molluscicides for the destruction of snails which act as intermediate hosts of human schistosomes. These snails are found in streams, irrigation ditches, swamps and lakes and are responsible for the wide incidence of bilharziosis in the Middle East, particularly Egypt.

This test has been developed especially for the field control of pentachlorophenates used for the destruction of these snails. It is necessary to determine the pentachlorophenate concentration in water at various localities. As these materials are effective at concentrations of 10 p.p.m., or less, the method has been developed to cover the range 5—100 p.p.m. It can, of course, be applied to higher concentrations provided that the sample is diluted prior to estimation. The equipment is portable, robust, and easy to use. After a short period of instruction even unskilled personnel should be able to analyse a water sample in about 5 minutes.

Principle of the method

It has been reported by Wallin[1], and by Haskins[2 3], that sodium and copper pentachlorophenates, in alkaline solution, will combine with methylene blue to form a chloroform-soluble blue complex. Over a limited range of concentrations it is possible to estimate the pentachlorophenate content of a water sample by forming this complex, extracting it with chloroform, and comparing the colour intensity of the chloroform extract with permanent glass standards.

Reagents required

1. Chloroform—analytical reagent grade
2. Sodium bicarbonate solution (20 g. analytical reagent grade in 100 ml. distilled water)
3. Sodium citrate—analytical reagent grade
4. Methylene blue—sodium bicarbonate reagent
   Dissolve three methylene blue tablets, each containing 19 mgm. of methylene blue chloride, or 0.046 g. of methylene blue chloride (87.5% assay) crystals, in 100 ml. of distilled water using a volumetric flask. Place 25 ml. of this solution in a 250 ml. separating funnel, add 50 ml. of saturated sodium bicarbonate solution followed by 50 ml. of chloroform. Shake the separator, allow the layers to separate, and reject the pink lower layer. Repeat the extraction, using 50 ml. portions of chloroform until the rejected layer is colourless. This usually requires about six additions of chloroform. Transfer the upper (aqueous) layer to an amber 125 ml. reagent bottle. This blue solution must be prepared freshly each day.

The Standard Lovibond Comparator Disc B.T. 400

The disc covers the range 5—100 p.p.m. of sodium pentachlorophenate. A dulling screen must be used with this disc. As the pentachlorophenate content of copper pentachlorophenate differs from that of the sodium salt by less than 3%, this disc may also be used for the determination of the copper salt without any significant loss of accuracy.

Technique

Take two clean, dry, stoppered graduated tubes (Lovibond 12 × 1.5 cm., S.T.P.T.C. acid wash tubes) and mark these A & B on the necks. Fill each tube to the first graduation with chloroform. Using a 5 ml. pipette fitted with a rubber bulb, obtain a sample for analysis by immersing the tip of the pipette into the water to be tested and squeezing and releasing the bulb three times before withdrawing the pipette. Add this sample to tube A, forming an aqueous layer above the chloroform and up to the second graduation.

Fill tube B in the same manner using water from the same source as the analytical sample to be tested, but taken before pentachlorophenate treatment. If this is not available then distilled water may be used. Add 1 ml. of the methylene blue sodium bicarbonate reagent, using a 1 ml. pipette fitted with bulb, to tubes A and B. Replace the glass stoppers in the tubes, shake both tubes for 30 seconds and then stand them upright for two minutes to allow the layers to separate. Eliminate any bubbles which are adhering to the inside of the tube by holding the tube almost horizontal and rotating it about its axis, (Figure 1).

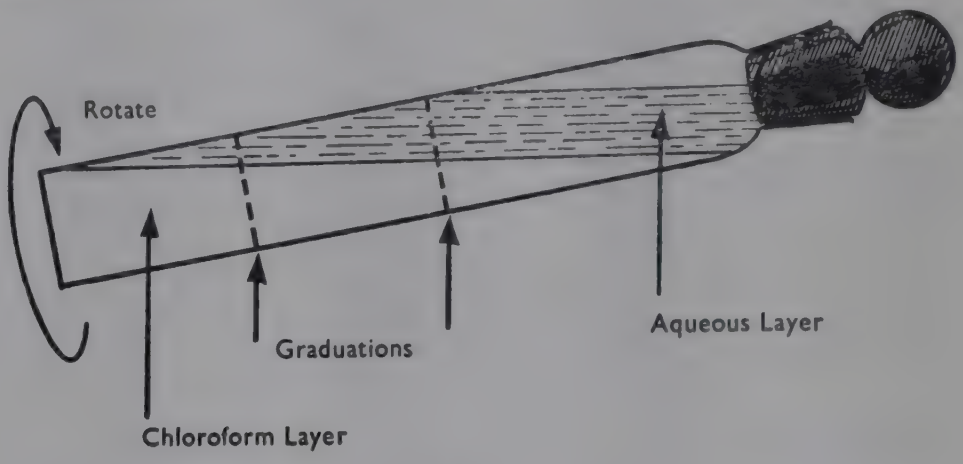


Figure 1

Place tube B in the left-hand compartment of the comparator. Examine the upper (aqueous) layer in tube A. If this is colourless, or only pale blue, add a further 1 ml. of the methylene blue reagent, shake for 30 seconds and allow to stand for two minutes. Repeat this operation until the upper layer is a definite blue. Having removed any bubbles, in the manner already described, place tube A in the right-hand compartment of the comparator. Make certain that the dulling screen is in position, and match the colour of the chloroform layer in tube A with that of the permanent glass standards in the disc using north daylight whenever possible (south daylight in the southern hemisphere). Note the disc reading. This is the sodium pentachlorophenate concentration in the sample in parts per million.

Notes

1. It will be noticed that the disc calibration ensures the greatest accuracy in the lower part of the range. A reading observed to be between 25 and 100 p.p.m. should always be confirmed by diluting the sample and repeating the test.

2. If the initial procedure yields a blue colour deeper than the 100 p.p.m. standard in the disc, the sample should be diluted with a known volume of distilled water, to bring the pentachlorophenate solution within the range of concentration covered by the disc. The diluted solution is used for the test and the answer obtained should be multiplied by the degree of dilution, (Table 1).

TABLE 1

Volume of water sample—ml.	Volume of distilled water added—ml.	Degree of dilution.
5	5	2
5	10	3
5	15	4
5	20	5
5	25	6
5	30	7
5	35	8
5	40	9
5	45	10

3. Water containing appreciable amounts of dissolved copper or iron may produce cloudy precipitates which are carried into the chloroform extract. This can be prevented by adding a few small crystals of analytical reagent grade sodium citrate to each sample of water before commencing the test.

4. The disc is calibrated with pure pentachlorophenate. Commercial grades of this material, e.g. Santobrite, contain small amounts of the salts of lower chlorinated phenols. These may also form blue complexes with the reagent but they do not significantly affect the accuracy of the test.

5. A complete kit (ref.: C7/215) for carrying out this test in the field may be obtained from:

Messrs. Baird & Tatlock (London) Ltd.,
Freshwater Road,
Chadwell Heath, Essex, England.

References

1. G. R. Wallin, *Anal. Chem.*, 1959, **22,** 1208
2. W. T. Haskins, *Pub. Health. Rep. (Washington)*, 1951, **66,** 33, 1047
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The Determination of Monohydric Phenols

using 4-amino-antipyrine

Introduction

The diazotised sulphanilic acid method described in this book for the estimation of phenol determines total phenols. The present method determines monohydric phenols only. It is thus possible, using a combination of both methods, to determine polyhydric phenols by difference.

The use of amino-antipyrine as a reagent for phenol was suggested by Emerson[1] and extensively investigated by Ettinger *et al*[2]. Procedures have been developed for the application of the test to fungicides[3], water and brine[4], and aqueous wastes from coke plants[5]. It has been found to be particularly suitable for trade effluent analysis, especially for gas-works liquors and for effluents in the phenolic plastics industry.

Principle of the method

The phenolic material is mixed with 4-amino-antipyrine and an alkaline oxidant in a solution of sufficiently high *p*H to prevent the formation of antipyrine-red. The oxidant normally used is potassium ferricyanide. The colour produced is compared with Lovibond permanent glass standards.

Reagents required

1. Hydrochloric acid, analytical reagent grade, diluted with an equal volume of water
2. Ammonia solution, 0.880, diluted with 8 volumes of water
3. 4-amino-antipyrine, 2% solution in water
4. Potassium ferricyanide, 8% solution in water
5. Buffer solution :—*p*H 10.0 — 12.370 g. of boric acid and 14.910 g. of potassium chloride dissolved and made up to 1,000 ml. with water. 250 ml. of this solution and 44 ml. of *N* sodium hydroxide are made up to 1,000 ml. with water.

The Standard Lovibond Comparator Disc 3/43

The disc covers the range 0.5-4.0 p.p.m. monohydric phenols, in steps of 0.5 p.p.m.

Technique

A suitable volume of the sample, containing not more than 400 micrograms of monohydric phenol, is placed in a 100 ml. Nessler tube or volumetric flask and made up to about 60 ml. with water. 10 ml. of buffer solution are added and the volume made up to about 90 ml. with water. The *p*H is checked and adjusted to between 9.6 and 10.0, if necessary, with the acid or alkali (reagents 1 and 2). Close range test papers are accurate enough for this check. The final volume is now adjusted to 100 ml. with water.

The solution is divided into two 50 ml. portions and 1 ml. of the antipyrine reagent is added to each portion. The solutions are mixed and a part of one is poured into a 13.5 mm. cell (or standard test tube) and placed in the left-hand compartment of the comparator to act as a blank. To the other 50 ml. of solution, 1 ml. of the ferricyanide reagent is added and, after mixing, a portion of the resulting solution is placed in a 13.5 mm. cell (or standard test tube) in the right-hand compartment of the comparator. Exactly 10 minutes after the addition of this reagent, the colour is matched against the disc, using a uniform source of white light—north daylight whenever possible.

The figure read from the indicator window represents p.p.m. of monohydric phenol in the final 100 ml. volume. From this figure the concentration in the original volume is calculated :—

$$\text{Concentration} = \frac{\text{Reading} \times 100}{\text{Original volume}} \text{ p.p.m.}$$

References

1. E. Emerson, *J. Org. Chem.*, 1943, **8,** 417
2. M. B. Ettinger *et al. Anal. Chem.*, 1951, **23,** 1783
3. S. Gottleib and P. B. Marsh, *Ind. Eng. Chem. Anal. Ed.*, 1946, **18,** 16
4. R. W. Martin, *Anal. Chem.*, 1949, **21,** 1419
5. J. A. Shaw, *Anal. Chem.*, 1951, **23,** 1788

The Determination of Total Phenols (Tar Acids)

using sulphanilic acid

Introduction

Phenols are found in gas liquor, coke-oven effluents, wastes from the production of phenol-formaldehyde resins, drainage from tarred roads, and in many chemical wastes. They are bactericidal and also very toxic to fish, and are therefore most undesirable in effluents discharged to rivers. If present in high concentrations, phenols may also produce a serious problem to sewage works using biological methods of purification.

In view of their presence in coal tar, phenols are often referred to as ' tar acids '.

Principle of the method

This test is based on the reaction between diazotised sulphanilic acid and phenols. The method adopted is that of Fox and Gauge[1] for the determination of tar acids in road dressings. As the tar acids present in road tar consist chiefly of higher phenols and xylenols which produce colours with diazotised sulphanilic acid ranging from yellow to red, it is not possible to make glass colour standards that will be perfect colour matches for the colours produced by different kinds of phenols. An arbitrary mixture of phenols has been used for making the solutions on which the glass colour standards are based, and generally these closely match the colours produced in testing aqueous extracts of road dressings and road drainage. This method determines total phenols. A different method is described in this book for monohydric phenols.

Reagents required

The following reagents, which must be of analytical reagent grade, are required :—

1. Sulphuric acid (1 in 4) prepared by mixing 1 volume of sulphuric acid with 3 volumes of water.
2. An 8% w/v aqueous solution of sodium hydroxide.
3. A 20% w/v aqueous solution of sodium hydroxide.
4. Chloroform.
5. Sulphanilic acid solution prepared by dissolving 1.91 g. of sulphanilic acid in 250 ml. of water.
6. Sodium nitrite solution prepared by dissolving 0.85 g. of sodium nitrite in 250 ml. of water.
7. Diazotised sulphanilic acid, prepared immediately before use, by mixing 5 volumes of the sulphanilic acid solution with 1 volume of sulphuric acid (1 in 4), cooling to 4°C. and adding slowly 5 volumes of the sodium nitrite solution.

The Standard B.D.H. Lovibond Nessleriser Disc NP

The disc covers the range from 0.01 to 0.09 part per 100,000, equivalent to 5γ to 45γ of tar acids in the 50 ml. volume used in the final colour matching.

Technique

Acidify a measured volume of the solution under examination—in the case of aqueous extracts of road dressings, 250 ml. is a suitable quantity—with 10 ml. of sulphuric acid (1 in 4) and extract three times with a total of 100 ml. of chloroform. Shake the combined chloroform extracts twice with 10 ml. of 20% sodium hydroxide solution diluted with an equal volume of water. Mix the alkaline solutions and dilute with water to 100 ml. (or other suitable quantity).

Transfer an aliquot portion of the alkaline solution to a Nessleriser glass, neutralize to litmus paper with sulphuric acid (1 to 4) and dilute to 40 ml. with water. Add 5 ml. of the diazotised sulphanilic acid and 5 ml. of 8% sodium hydroxide solution, mix thoroughly, allow to stand for five minutes and place in the right-hand compartment of the Nessleriser. In the left-hand compartment of the instrument place another Nessleriser glass containing the same

quantities of the alkaline solution and sulphuric acid, together with sufficient water to produce 50 ml. Stand the Nessleriser before a uniform source of white light—a north window is the best—and compare the colour produced in the test solution with the colours in the standard disc, rotating the disc until the nearest colour match is obtained. Should the colour in the test solution be deeper than the standard colours, a fresh test should be conducted, using a smaller quantity of the alkaline solution.

The markings on the disc indicate the concentration of phenols in parts per 100,000 in the 50 ml. of solution contained in the Nessleriser glass, from which, by simple arithmetic, the concentration in the original solution may be ascertained.

Notes

1. It must be emphasized that the readings obtained by means of the B.D.H. Lovibond Nessleriser and disc are only accurate provided that Nessleriser glasses are used which conform to the specification employed when the discs were being calibrated, namely that the 50 ml. calibration mark shall fall at a height of 113 mm., plus or minus 3 mm. measured internally.
2. The preparation of the necessary disc for the above test was suggested by members of the staff of the Ministry of Agriculture and Fisheries at Alresford, Hampshire, and the method was worked out by them in conjunction with The Tintometer Ltd.
3. To convert to parts per million, use formula $\frac{\text{Disc reading}}{\text{Volume used}} \times 500$

Reference

1. J. J. Fox and A. G. H. Gauge, *J.S.C.I.*, 1920, **39**, 260T; 1922, **41**, 173T

The Determination of Pyridine

using copper silicomolybdic acid

Introduction

The method developed by Sutherland, Daroga and Pollard[1] for the determination of nicotine has been modified by Daroga and Pollard[2] and extended to the determination of pyridine. This method has been used for the determination of pyridine in air, in soil and in methylated spirit.

Principle of the method

Pyridine is precipitated as a complex with copper-silicomolybdic acid reagent. The pyridine silicomolydbic complex is reduced to the blue molybdenum compound by means of sodium sulphite and glycine. The blue colour is compared with Lovibond permanent glass standards using either a Tintometer or a B.D.H. Lovibond Nessleriser[2].

Reagents required

1. Copper-silicomolybdic acid

Molybdic anhydride	14.4 g.
Sodium hydroxide (Normal solution)	100 ml.
Silica (as sodium silicate solution)	0.7 g.
Hydrochloric acid—10% solution	
Copper chloride	2.5 g.

The molybdic anhydride is dissolved in the caustic soda, by warming, and the sodium silicate is added. Hydrochloric acid is next added in small quantities, with stirring, until the solution becomes green. The solution is now diluted to 900 ml. and heated on a boiling water-bath for 3 hours. It is then allowed to cool and to stand for 24 hours at ambient temperature. Any excess silica is then filtered off and the copper chloride, dissolved in a little water, is added to the filtrate. This is then diluted to 1 litre and stored in a dark bottle. The reagent is stable for three months.

2. Wash liquid

10% sodium chloride in 0.1N. hydrochloric acid

3. Reducing solution

Glycine	1 g.
Sodium sulphite (20% aqueous solution)	15 ml.
Ammonium hydroxide (d 0.880)	5 ml.

The glycine is dissolved in the sodium sulphite solution, and the ammonium hydroxide added slowly with stirring. The solution is diluted to 100 ml.
This reagent is only stable for 24 hours and should be freshly prepared each day.

The Standard B.D.H. Lovibond Nessleriser Disc NOG

The disc covers the range 0.6 to 4.5 mg. of pyridine.

Technique

1 ml. of reagent 1 is added to 1 ml. of the sample and 1 ml. of 0.1 N-hydrochloric acid in a 30 ml. beaker. The mixture is warmed and agitated until the precipitate coagulates. The precipitate is allowed to stand for at least an hour at ambient temperature and is then collected on a disc of No. 42 Whatman filter-paper in a small (No. 00) Gooch crucible. The beaker and precipitate are washed twice with successive 0.5 ml. portions of reagent 2. The filter flask is then emptied and washed.

1-2 ml. of reagent 3 are pipetted into the beaker to dissolve any residual precipitate. The solution is then poured into the Gooch crucible and the precipitate dissolved. The solution is then sucked into the filter flask, and the beaker and filter are washed with more reagent 3. The

combined liquid and washings from the filter flask are transferred to a 50 ml. graduated flask and diluted to the mark. The flask is heated in a water-bath at 45°C. for 25 minutes. After cooling, the solution is transferred to a standard Nessleriser tube which is placed in the right-hand compartment of a B.D.H.-Lovibond Nessleriser, and compared with the colours in the standard disc using north daylight as the illuminant.

Notes

1. The procedure must be followed exactly, as it has been shown that the temperature of colour development, the acid concentration during precipitation, and the time elapsing between precipitation and filtration all affected the colour produced.

2. The readings obtained by means of the B.D.H. Lovibond Nessleriser and disc are only accurate provided that standard Nessleriser tubes are used, in which the 50 ml. calibration mark falls at a height of 113 ± 3 mm. measured internally.

References

1. G. L. Sutherland, R. P. Daroga and A. G. Pollard, *J.S.C.I.*, 1939, **58**, 284
2. R. P. Daroga and A. G. Pollard, *J.S.C.I.*, 1941, **60**, 207

The Determination of Sugar

using α-naphthol

Introduction.

The quantitative determination of small traces of sugar in water is of considerable importance to Sugar Manufacturers and in all factories where sugar is handled. If the sugar content in boiler water exceeds 50 p.p.m., corrosion of the boiler is almost certain to ensue. This contamination of the boiler water may be the result of returned condensates, or wash waters used in boiler feed, which give a zero polarimetric reading.

Sugar losses in effluent waters may also be determined by this same method.

Principle of the method

Sugar reacts with α-naphthol and sulphuric acid to give a violet colour, which may be used for the estimation of sugar in dilute solutions[1]. The method has been applied to the determination of glucose in blood[3] and to other dilute sugar solutions[2].

Reagent required.

0.8 g. dry α-naphthol (analytical reagent grade) in 100 ml. pure concentrated sulphuric acid (98%)

This reagent must be made fresh daily.

The Standard Lovibond Comparator Disc 3/29

This disc contains colour standards for 10, 25, 50, 75 and 100 parts per million of sugar.

Technique

If the water to be tested contains much organic matter it should be filtered before testing.

Pour 2.5 ml. of the water to be tested into the glass-stoppered test tube (see Note 4), and pipette down the side of the tube 5 ml. of the α-naphthol solution in sulphuric acid. Place the stopper in the tube and mix gently by inverting the tube three times, firmly holding the stopper in position. Allow to stand for exactly ten minutes with the stopper removed. Replace the stopper, and place the tube in the right-hand recess of the comparator. Revolve the disc until the colour standard matches the solution, and then read off the amount of sugar in parts per million in the indicator recess.

Concentrations of sugar over 100 p.p.m. may be determined by simple dilution, as for example when estimating sugar or syrup losses via effluent water to drain or river.

Notes

1. Tests should be made on fresh or recently obtained samples only, as the sugar content of samples several hours old would be greatly reduced due to bacterial decomposition.

2. Considerable heating takes place on the addition of the acid solution, and the stopper should be removed each time the tube has been inverted. Hold well away from the face when inverting the tube, and always handle the acid with great caution.

3. The colours must be matched exactly ten minutes after the addition of the acid, because the colour does not fully develop until this time has elapsed, and shortly after begins to change.

4. A special test tube with a ground glass stopper must be used with this test, to avoid the risk of acid burns during mixing. Suitable 13 mm. tubes with ground-in stoppers may be obtained from Tintometer Ltd.

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The Determination of Sunconox 18

(N–stearoyl–*p*–amino–phenol) using nitrous acid

Introduction

Sunconox 18 is widely used as an antioxidant in plastics, rubbers, oils, etc. When incorporated into polythene which is to be used for the construction of piping for cold water services, the concentration of Sunconox 18 must fall within the limits laid down in the appropriate British Standard[1]. This test has been developed especially for this purpose, but can be applied to the determination of Sunconox 18 in other materials provided that a suitable extraction procedure is adopted.

Principle of the method

N-stearoyl-*p*-amino-phenol (Sunconox 18) reacts with nitrous acid to give a yellow nitroso derivative, the colour intensity of which is proportional to the amount of Sunconox 18 present. This colour is measured by comparison with a series of Lovibond permanent glass colour standards.

Reagents required

All reagents must be of analytical reagent quality, and distilled water must be used whenever water is specified.

1. Toluene — sulphur free
2. Tetrahydrofuran — freshly distilled
3. Potassium nitrite 10% (w/w) solution in water
4. Acetic acid 10% (w/w) solution in water

Standard Lovibond Discs, Comparator Disc 3/61 and Nessleriser Disc NOL

Disc 3/61 covers the range 0.02% to 0.3% Sunconox 18 and is designed to be used with the Lovibond Special Purposes Comparator and 40 mm. cells. Disc NOL covers the range 0.002% to 0.05% Sunconox 18.

Technique

Dice a sample of polythene into cubes of edge less than 1 mm. and weigh 1 g. of this diced material into a 100 ml. boiling flask. Add 18 ml. of toluene from a pipette and heat, under a reflux condenser, on a boiling water-bath. Swirl the flask periodically until all the polythene has dissolved. Solution is usually complete within 30 minutes. Add 20 ml. of tetrahydrofuran by means of a pipette and continue the refluxing for a further 30 minutes. While still hot, decant the solution and the precipitated polythene into a beaker and allow to cool. Filter the cold liquid into a 250 ml. distillation flask and expel as much liquid as possible from the gelatinous precipitate by pressing. Return the precipitate to the boiling flask and repeat the extraction process as before. Filter. Finally wash out the condenser and the boiling flask with two 12 ml. portions of tetrahydrofuran and add these to the filtrate. Concentrate the filtrate to approximately 10 ml. by distilling off excess toluene under reduced pressure, and allow to cool. Transfer the concentrated extract to a 50 ml. volumetric flask. Wash the distillation flask with two successive 5 ml. portions of tetrahydrofuran, add the washings to the concentrate and dilute to 50 ml. with toluene. This final solution should be clear and colourless.

Transfer this 50 ml. to a separating funnel, add 5 ml. of potassium nitrite solution (reagent 3) followed by 5 ml. of acetic acid solution (reagent 4). Shake for 5 minutes and then stand for 40 minutes to allow the layers to separate. Discard the lower (aqueous) layer. Filter the yellow toluene layer into a Nessler tube and place this in the right-hand compartment

of the Nessleriser. Prepare a blank solution by mixing 40 ml. of toluene, 10 ml. of tetrahydrofuran, 5 ml. of potassium nitrite solution and 5 ml. of acetic acid solution. Fill an identical Nessler tube to the 50 ml. mark with this solution and place it in the left-hand compartment of the Nessleriser. Compare the yellow colour of the sample solution with the Lovibond permanent glass standards in the disc, using north daylight wherever possible. The figure read off from the disc gives directly the percentage of Sunconox 18 in the sample of polythene.

If the colour of the sample solution is too intense to be matched by the Nessleriser disc, transfer the sample and blank solution to 40 mm. cells, and place these in the right-hand and left-hand compartments respectively of a Lovibond Special-Purposes Comparator. Compare the colour with the standards in the disc as before and read off the percentage of Sunconox 18 as in the case of the Nessleriser determination.

Notes

1. It is essential that exactly 1 g. of diced polythene be used for the test if the disc readings are to give the percentage of Sunconox 18 directly.

2. Readings obtained by the use of a B.D.H. Lovibond Nessleriser and disc are accurate only provided that Nessler cylinders are used which are identical with those used when the discs were calibrated, that is cylinders in which the 50 ml. mark falls at a height of 113 mm. ± 3 mm. measured internally.

Reference

British Standard 3284 : 1961 *"Specification for Polythene Pipe (Type 710) for cold water service."* 1961, London, British Standards Institution.

The Determination of Thiophen

using alloxan

Introduction

This method has been developed for determining the purity of benzoles as regards the thiophen content, and covers the range .0002% to .0025%. This disc has been adopted by the S.T.P.T.C. under Serial L.B. 21—57 and B.S. 135: 1953. Higher quantities may be determined by diluting the sample with thiophen-free benzene.

Principle of the method

The sample of benzole is shaken with a solution of alloxan in sulphuric acid, and the colour produced is compared with the standard colours in the disc, which was prepared, in conjunction with the National Benzole Association Research Department, by carrying out the test on benzene solutions containing known quantities of thiophen.

Reagents required

A solution containing 0.01% (w/v) alloxan dissolved in sulphuric acid containing 90.5% ± 0.5% (w/w) pure sulphuric acid, checked by titration.

This reagent shall be used within 7 days of its preparation.

The Standard Lovibond Comparator Disc 3/19

The disc covers the range 4γ to 20γ (0.004 to 0.02 mg.) of sulphur (S), present as thiophen.

Technique

Prior to the test, the sample and reagent must be brought to a temperature between 15° C. and 20° C.

Place 5 ml. of the reagent in a stoppered test tube as specified below (Note 4). Add 2ml. of the sample. Stopper firmly and shake for exactly one minute at the rate of 100—120 shakes per minute. Place in a water bath at 15° C.—20°C. for exactly 30 minutes, at the end of which time transfer the tube to the right-hand compartment of the comparator and match against the disc by north daylight.

The figure shown represents micrograms of sulphur in the 2 ml. sample taken.

If the colour produced is greater than the highest standard, a smaller quantity of sample must be taken and diluted to 2 ml. in the thiophen-free benzene.

Notes

1. No temperature control is necessary when the laboratory temperature is between 15° C. and 20°C.

2. The lower limit of the test cannot be reduced by using a greater proportion of sample to reagent.

3. Thiophen-free benzene may be prepared by refluxing pure benzole for nitration[3] with 50% v/v of a 20% w/v aqueous solution of mercuric acetate containing 7.5 ml. of glacial acetic acid per 100 ml. of solution. After washing with water, the sample must be refractionated through a 12 pear column, the fraction distilling at 80°-80.5° C. being collected. Five millilitres of this benzene, shaken with 5 ml. of the alloxan reagent described above, must be allowed to stand for 30 minutes. The colour produced in the acid layer must not be darker than a freshly prepared solution containing 0.2 gms. of potassium dichromate in 1000 ml. of a mixture of distilled water and sulphuric acid of 98% w/w strength, as described in S.T.P.T.C. Serial No. L.B. 10—57.

4. The stoppered test tube used must conform to the specification for the National Benzole Association and British Standards Institution Acid Wash Test on low boiling fractions of coal tar, except that it does not require any calibration mark. Suitable test tubes 13 mm. dia. with ground glass stoppers may be obtained from Tintometer Ltd.

References

1. K. H. V. French, *J.S.C.I.*, 1946, LXV, 15
2. S.T.P.T.C., "*Standard Methods for Testing Tar and its Products*", 1957
3. *N.B.A. Specification No.* 2, 1938
4. *British Standard*, 135: 1953

The Determination of Vitamin A

using antimony trichloride

Introduction

This method, popularly known as the "Blue Value" or "Blue Units" method, was approved in 1931 by the British Pharmacopœia Commission, Cod-liver Oil Colour Test Sub-committee, and is still in wide use. With the necessary modifications, it may be used for materials other than cod-liver oil.

The test was designed for use with the Lovibond Tintometer.

Principle of the method

A solution of antimony trichloride, in pure dry chloroform, reacts with Vitamin A to produce a bright blue colour. This colour, which quickly develops and then at once begins to fade, is compared with permanent glass standards in a Lovibond Tintometer.

Reagent required

A solution of antimony trichloride in pure dry chloroform saturated at 20°C. is prepared in the following way:

Wash chloroform two or three times with its own volume of distilled water, dry the chloroform over anhydrous potassium carbonate; pour off and distil, rejecting the first 10 per cent. of the distillate. During drying and distillation protect the chloroform from light.

Wash antimony trichloride with the pure dry chloroform until the washings are clear. Prepare a solution, saturated at 20°C., of the washed antimony trichloride in the pure dry chloroform. The solution, which must contain not less than 21 and not more than 23 per cent. w/v of $SbCl_3$, should be kept in a well-stoppered bottle of amber-coloured glass.

Assay.—Mix 1 ml. with a solution of 2 g. sodium potassium tartrate in 20 ml. of water; rotate the mixture, add 2 g. sodium bicarbonate and titrate with *N*/10 iodine. Each ml. of *N*/10 iodine is equivalent to 0.01141 g. of $SbCl_3$.

Technique

Dissolve 2 g. of the oil, accurately weighed, in sufficient pure chloroform at 20°C. to produce 10 ml. Place 0.2 ml. of this solution (equivalent to 0.04 g. of the oil), measured accurately from a 1 ml. burette graduated in 1/100ths. ml., in a 1 cm. Tintometer cell, and place this cell in position for viewing in the Tintometer White Light Cabinet.

Add rapidly 2 ml. of the antimony trichloride reagent to the oil in the cell, so that the two solutions mix. The blue colour, which develops in intensity and then fades, is matched by means of the standard Tintometer glasses **at the point of maximum intensity.** It may be necessary to carry out several tests before an accurate reading is obtained, and it may also be necessary to use red or yellow glasses, in addition to the blue glasses, or to superimpose Neutral Tint glasses over the sample, in order to obtain an accurate colour match. The result is recorded as the number of blue units required to match the colour developed from 0.04 g. of the oil. Any red or yellow colours or Neutral Tints that it may be necessary to use in the colour matching are ignored, and the blue only is recorded.

In the case of a cod-liver oil of high value, it will be necessary to use a more dilute solution of the oil in chloroform. The concentration and the colour production do not form a straight line graph and strictly comparable results are only obtained when the colour matched lies between 4.0 and 6.0 Lovibond units. Hence, it is essential to adjust the strength of the chloroform solution so that the amount of blue required lies between these values. The proportionate value for 0.04 g. is then calculated. This value is known as the Carr-Price value, and may be defined as follows:—

The Carr-Price value is the proportionate amount of blue colour, expressed in accordance with the Lovibond scale, which is developed by 0.04 g. of the oil or substance when 0.2 ml. of a chloroform solution of the substance is treated with 2 ml. of a saturated solution of antimony trichloride in dry alcohol-free chloroform, the concentration of the solution of the substance being so adjusted that the colour produced, matched and measured in a 1 cm. cell in a Lovibond Tintometer at the point of maximum intensity, lies between the limits of 4.0 and 6.0 blue, and preferably between the limits of 4.8 and 5.2.

This Carr-Price blue value may be converted into International Units per gram (British computation) by multiplying by 32, but for refined whale products it is considered that the factor 23 gives figures more in line with the biological results.